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DISEASES OF CHILDREN

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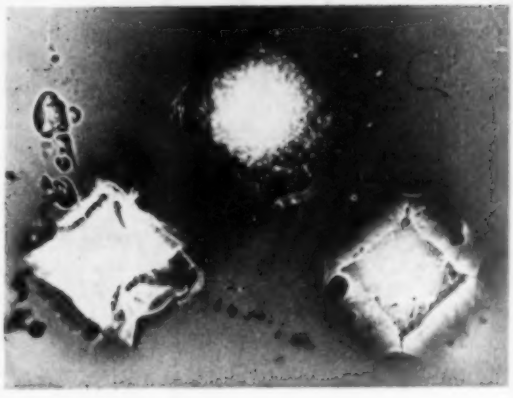

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A.M.A. Journal OF
DISEASES of CHILDREN

Transient Dysproteinemia in Infants, A New Syndrome

I. Clinical Studies

Robert A. Ulstrom, M.D.
Nathan J. Smith, M.D.
and
Ernest M. Heimlich, M.D., Los Angeles

Within an 18-month period four infants have been seen with the unusual clinical findings of edema, pallor, and marked irritability. Preliminary laboratory studies have demonstrated hypoproteinemia and anemia in the absence of other signs of hepatic or renal disease in each patient. Although similar clinical situations have been discussed previously in pediatric writings,* none of the reported patients appears to have had the same clinical syndrome as those to be described here. For this reason the clinical findings in the four patients will be presented in some detail, and the many laboratory investigations undertaken in an attempt to define pathogenesis of the hypoproteinemia and anemia will be discussed.

Submitted for publication June 14, 1956.

John and Mary R. Markle Scholar in the medical sciences; present address, Department of Pediatrics, University of Minnesota Hospitals, Minneapolis (Dr. Ulstrom); assistant Professor of Pediatrics, University of California School of Medicine (Dr. Smith), and Instructor in Pediatrics, University of California School of Medicine (Dr. Heimlich).

*References 1-5.

Report of Cases

Case 1.—This Caucasian girl was born on Nov. 15, 1953, after an uneventful pregnancy and normal labor. Her birth weight was 3600 gm. (7 lb. 15 oz.). She was first seen in the Department of Pediatrics of the University of California at Los Angeles at one month of age. At this time, the mother recognized no specific problems and was feeding the infant an evaporated milk formula. At 5 days of age she had been given a synthetic vitamin C supplement which was thought to have caused a papular erythematous eruption on the face. Orange juice had been substituted, and the rash disappeared. Physical examination at one month of age failed to reveal any abnormalities. Her weight at that time was 4800 gm. (10 lb. 9 oz.) and length 59.7 cm. (23½ in.). The family history revealed that the parents were healthy. A maternal half-sister and one brother were living and well.

The patient was seen at monthly intervals for the next five months. Her weight gain was good. Fruits, cereal, vegetables, meats, and egg yolk were added to the diet beginning at 2 to 4 months of age. Motor and social development were normal. Diphtheria, pertussis, and tetanus immunizations were given at monthly intervals starting at 3 months of age, and a smallpox vaccination resulted in an uncomplicated primary vaccination reaction at 5 months. At 6 months of age, the baby was examined and found to have a mild respiratory infection. Phenylephrine (Neosynephrine) hydrochloride nose drops were prescribed. Her weight at 6 months of age was 8400 gm. (18 lb. 8 oz.)

At 7½ months of age, the patient was seen because of marked irritability, periobital and facial swelling of one week's duration, and increasing size of the abdomen and legs. On examination, a

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moderate degree of generalized edema was noted. The liver edge was palpable 3.5 cm. below the right costal margin. Her appetite had remained good, and the food intake was normal. Her weight was 9200 gm. (20 lb. 4 oz.).

At this time, the total serum proteins were 3.0 gm. per 100 cc. with 1.1 gm. per 100 cc. globulin and 1.9 gm. per 100 cc. albumin. The nonprotein nitrogen in the plasma was 11.1 mg. per 100 cc. The patient was hospitalized on July 12, 1954, and observed for a period of 25 days.

Initial diagnostic efforts were directed at more precisely defining the nature of the hypoproteinemia. The serum total protein level on admission was 2.8 gm. per 100 cc. Tiselius electrophoresis of the plasma proteins soon after admission on July 14, 1954, demonstrated low normal levels of alpha and beta globulins and a normal level of fibrinogen. Gamma globulins could not be demonstrated, and the albumin was reduced. Total serum proteins on this date had risen to 3.8 gm. per 100 cc. with 1.8 gm. of albumin and 2.0 gm. per 100 cc. globulin. On July 24, 1954, the total serum protein level was 4.6 gm. per 100 cc. and remained at approximately this level during her hospitalization.

Striking changes in the patient's weight occurred as the serum proteins increased in concentration. The weight which was 9500 gm. (20 lb. 14 oz.) on admission, fell to a low level of 8370 gm. (18 lb. 7 oz.) during the first six days of observation. The obvious edema disappeared, and the output of urine was distinctly increased.

A dietary balance study to evaluate the over-all metabolism of nitrogen, fat, phosphorus, and potassium was begun on July 20, 1954, the eighth hospital day, and was continued for a period of three days. The results of this study are to be discussed later.

The hemoglobin concentration at the time of admission was 7.8 gm. per 100 cc. During the first 10 days of hospitalization, the hemoglobin fell to 4.9 gm. per 100 cc., owing in part to the repeated blood loss necessitated by many laboratory studies. During this time, the platelet counts were normal and a moderate lymphocytosis was found in studies of the leucocytes. The erythrocyte MCV was reduced, as was the MCHC. On July 20, 1954, the serum iron level was 32.1γ per 100 cc., the serum copper level was elevated, and the free erythrocyte protoporphyrin 33.2γ per 100 cc. The total iron binding capacity of the serum was reduced to 221γ per 100 cc. A bone-marrow aspiration biopsy was obtained and revealed normal granulopoietic elements and megakaryocytes. The erythroid elements were remarkable in that it was impossible to identify any specific normoblastic hyperplasia which is to be expected in infants with iron depletion. A normal number of plasma cells were found in the marrow specimen.

Although these findings seem to indicate the patient was suffering from iron depletion, the patient's age, history of a good diet, low free-erythrocyte-protoporphyrin level, low total iron binding capacity of the serum, and absence of normoblastic hyperplasia in the marrow were not in keeping with this conclusion. To further evaluate the iron metabolism, a dose of 200 mg. of saccharated iron oxide was given intravenously on July 26, 1954. This was followed by a prompt and progressive rise of hemoglobin concentration over the next eight weeks.

During the period of hospital study, it was not possible to define abnormalities in renal or hepatic function. Urinalysis on many occasions was normal. There was no azotemia. The cephalin flocculation test was negative. The serum bilirubin, thymol turbidity, and serum cholinesterase were low.

On Aug. 6, 1954, after 25 days hospitalization, the patient was discharged. She was free of clinical edema, weighed 9120 gm. (20 lb. 1 oz.), and the serum protein level was 4.8 gm. per 100 cc.

The patient was followed in the outpatient clinic, and when seen at 10 months of age she was free of symptoms other than some pallor. There was no hepatomegaly. The serum total protein level was 6.57 gm. per 100 cc. with a normal A/G ratio. The hemoglobin level was 11.6 gm. per 100 cc. There was a rather striking lymphocytosis noted at this time.

Because of the very low gamma-globulin level found early in the course of this girl's illness an attempt was made to evaluate her ability to produce antibodies. Two attempts to repeat the smallpox vaccination at the site of the previous primary vaccination, after edema had disappeared, failed to produce any reaction. A third vaccination resulted in a typical immune reaction. Her serum was found to contain normal concentrations of tetanus and diphtheria antitoxin, and Hemophilus pertussis antibodies. The Schick test was negative. The serum mucoprotein was 0.20 mg. per 100 cc. The blood type was O CDe/cde M/M. with both Anti-A and Anti-B agglutinins present.

At one year of age, the patient was free of any symptoms. She weighed 9900 gm. (21 lb. 12 oz.) and was 74.8 cm. (29½ in.) in length. Her diet and appetite were excellent. Laboratory studies showed only a slight lymphocytosis and a hemoglobin level of 10.6 gm. per 100 cc.

At 16 months of age (March 22, 1955) she became acutely ill with hyperpyrexia, and a diffuse papular rash which became hemorrhagic. She was hospitalized. A diagnosis of meningococcemia was made, and confirmed by bacteriologic studies of the blood and skin lesions. Her response to therapy of penicillin, sulfadiazine, and hydrocortisone was prompt, and she was discharged from the hospital

after nine days. Serum protein studies, including electrophoretic analysis, were normal.

During the remainder of her second year, the patient continued to enjoy good health except for episodes of acute respiratory disease. These infections were of short duration and produced no sequelae. At 2 years of age, she weighed 13.3 kg. and was 87.5 cm. in length. The serum proteins were normal, the hemoglobin level 12.7 gm. per 100 cc.; the serum cholinesterase was elevated (1.75ΔpH).

At the present, at 27 months of age, she continues to be free of symptoms. Recently her protein metabolism has been further studied after the intravenous administration of a dose of C^{14}-labelled phenylalanine.

CASE 2.—This Caucasian boy was born at term on May 17, 1954, after a normal pregnancy and delivery. He was first seen by us at 6 months of age because of facial swelling, pallor, and irritability.

The history revealed that he had enjoyed good health until 4 months of age, except for a mild respiratory infection at 2 weeks of age. The diet had been excellent, and was taken well. At four months, he had experienced a severe respiratory infection which was followed by the appearance of poor appetite and swelling about the eyes. Application of an ointment to the eyelids was thought to have caused some temporary improvement, but the swelling was even more marked by 5½ months. With the reappearance of the facial swelling, the irritability and refusal to take feedings increased. Some vomiting and occasional diarrhea stools were noted during this two-week period prior to hospitalization.

When seen at 6 months, he weighed 7850 gm. (17 lb. 4 oz.), was pale, and markedly edematous. Ascites was present. No hepatomegaly could be indentified. The serum total protein level was 2.6 gm. per 100 cc., with 1.7 gm. albumin per 100 cc. and 0.9 gm. globulin per 100 cc. Study of the serum by Tiselius electrophoresis revealed all globulin components to be uniformily reduced. The hemoglobin concentration was 5.0 gm. per 100 cc., with 13,500 leucocytes per cu. mm., 61% of which were lymphocytes. The serum iron, and copper, and iron binding capacity of the plasma were all reduced. The free erythrocyte protoporphyrin content was elevated.

The urinalysis, Addis count, thymol turbidity, prothrombin time, serum cholesterol, blood urea nitrogen, blood sugar, and serum electrolyte studies were all normal. No abnormalities were seen in x-rays of the chest, abdomen, and entire skeleton.

The patient was fed a normal diet for age in addition to homogenized milk. This was taken well. While he was being observed, the total proteins rose to 3.6 gm. per 100 cc. in one week. During this time he lost nearly 1800 gm. (4 lb.) of body weight.

After one week of study, the patient was transferred to another hospital where he was observed further. Twelve days after being seen by us initially, the total serum protein level was 4.3 gm. per 100 cc. Edema had largely disappeared, and the patient was eating well. The hemoglobin level had fallen to 4.0 gm. per 100 cc., and the reticulocyte count was 2.0%. After the ascites had disappeared, the liver edge was palpable 2.0 cm. below the costal margin.

While the hemoglobin level remained very low (4.0 to 5.0 gm. per 100 cc.) the plasma protein concentration continued to rise, reaching a level of 5.0 gm. per 100 cc. in another 10-day period of observation. The patient was given iron medication orally, starting on the 20th day of observation. There was a prompt and optimal reticulocyte response and subsequent rise of the hemoglobin level to 10.4 gm. per 100 cc.

After 34 days of hospital observation the patient was discharged but readmitted after 5 days because of a moderately severe respiratory infection. At the time of readmission, the serum protein level was 6.35 gm. per 100 cc. and the hemoglobin, 10.4 gm. per 100 cc. Response to symptomatic therapy was satisfactory. He was discharged from the hospital the sixth day and has been lost to subsequent follow-up study.

CASE 3.—This Caucasian girl was born on March 1, 1955, after a normal pregnancy and labor. She weighed 2500 gm. (5 lb. 8 oz.) at birth. During the first week of life, the child cried excessively. On the seventh day, she had a generalized convulsion associated with a body temperature of 106 F. The patient was hospitalized for a period of seven days and was thought to have had severe gastroenteritis. Treatment consisted of parenteral fluids and antibiotics.

A number of formula changes were made during the two-week period following discharge from the hospital because of diarrhea and failure to take feedings well. At one month of age an unusual degree of persistent crying and irritability began again. The patient was said to cry 10 to 14 hours without interruption. During this period she had five to seven small soft stools daily. At 2 months of age, she began to regurgitate small amounts of each feeding.

At 9 weeks of age, she was seen for the first time in the University of California at Los Angeles Clinic. The history revealed that she was the only child in this family, and both parents were living and well.

On physical examination, it was found that the patient weighed 4800 gm. (10 lb. 9 oz.), was 59.1 cm. (23½ in.) in length. No abnormalities were evident. The liver was not considered enlarged.

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For a period of four weeks she was treated only with small doses of sedatives. These produced significant symptomatic improvement. When sedation was not given, extreme irritability was present. A neurologist failed to find any abnormality. The electroencephalogram was not remarkable. The urinalysis was normal, and at 3½ months of age, the hemoglobin level was 7.4 gm. per 100 cc. Two weeks later, the hemoglobin had fallen to 5.8 gm. per 100 cc., the reticulocyte count was 2.5%, and the leucocytes and platelets were normal.

There appeared to be no obvious explanation for the rapidly developing anemia at this age. A bone-marrow aspiration was performed and revealed only a moderate reduction in erythroid precursors. The patient was hospitalized at 4½ months of age for study. She had continued to be very irritable. She passed five to seven small, foul stools daily and took feedings with some difficulty.

On physical examination it was noted that the patient was very pale and facial edema was present. The liver edge was palpable 3.0 cm. below the costal margin, and the spleen tip 5.0 cm. below the left costal margin.

Early studies during the patient's hospitalization were directed at determining the cause of the edema and anemia. The serum total protein level was 2.7 gm. per 100 cc. with 1.3 gm. of albumin per 100 cc. and 1.4 gm. of globulin per 100 cc. Tiselius electrophoretic analyses of the plasma proteins demonstrated a uniform reduction in the albumin and globulin components. Gamma globulin was completely absent in the tracings. Fibrinogen was present in normal concentration. The hemoglobin level was 4.6 gm. per 100 cc., the hematocrit 14.3%, and erythrocyte count 1,940,000/cu. mm. There was a leucocytosis with a distinct lymphocytosis noted. The platelet count was normal. The serum iron and copper levels were markedly reduced. The free-erythrocyte-protoporphyrin level was elevated. Another aspiration of the marrow showed 23% erythroid precursors, a definite increase in lymphocytes, and normal granulopoietic elements.

During the first few days of study, a variety of diagnostic tests was made; all gave normal findings. These included repeated urinalysis, blood sugar, alkaline phosphatase, serum cholesterol, serum bilirubin, sulfobromophthalein clearance, clotting time, prothrombin time, prothrombin consumption test, blood urea nitrogen, x-rays of the skull and entire skeleton, and x-rays of the upper and lower gastrointestinal tract with barium and air. Findings at protoscopy were normal.

In studying the patient's ability to produce antibodies, it was found that she was blood type A, with an anti-B antibody titer of 1:8. Later in the course of the studies, normal antibody response to diphtheria and tetanus immunization was demonstrated.

During her prolonged hospitalization, there was no significant alteration in the very low serum protein level, but there was some variation in the degree of edema. The hemoglobin level fell to 2.9 gm. per 100 cc. A prompt reticulocytosis followed the oral administration of iron with a very remarkable rise in hemoglobin to 11.0 gm. per 100 cc. in three weeks. The lymphocytosis persisted.

The patient was fed a normal infant diet while in the hospital. This consisted of homogenized milk, cereal, vegetables, fruits, and meat. Her calculated caloric intake was approximately 130 calories per kilogram of body weight. In a representative 24-hour period she ingested 10.5 gm. of carbohydrate, 5.8 gm. of protein, and 5.7 gm. of fat per kilogram of body weight. Owing to the many laboratory studies, her hospital dietary intake was irregular at times, especially early in the course of her studies. Her weight which was 7600 gm. on admission, fell to 5820 gm. in twelve days, and had increased to 6600 gm. at the time of discharge. The head circumference increased from 38.0 cm. to 39.5 cm. during this eight-week period.

At 6½ months of age, she was discharged from the hospital. Edema and hypoproteinemia were still present. The hemoglobin level was 10.5 gm. per 100 cc. She was eating well and was less irritable than when admitted. Iron and vitamin supplementation was continued at home.

After 13 days at home, the patient was again examined. Her dietary intake at home had been excellent. She had been extremely irritable, edema had increased, and she had had symptoms of a mild respiratory-tract infection. The patient was again admitted to the hospital for study. The serum protein level was 2.3 gm. per 100 cc., and, although the patient had continued to take iron at home, the hemoglobin was only 9.2 gm. per 100 cc. The leucocyte count on admission was 13,820, with 35.5% neutrophiles, 35.5% lymphocytes, and 18.0% mature eosinophiles. Alpha and beta hemolytic streptococci were found in the nasopharyngeal culture.

On physical examination, the most striking finding was the facial edema and pitting edema of the lower extremities. She weighed 7800 gm., was 69 cm. in length, and the head was 40.0 cm. in circumference. The body temperature was 100 F (37.8 C). Slight inflammation of the nasopharyngeal mucous membranes and tympanic membranes was present. Treatment with a triple sulfonamide preparation was given, and the symptoms of the mild respiratory-tract disease cleared in the following five days. Supplementation with iron medication was continued in the hospital.

On the 11th hospital day, the serum protein level was 2.8 gm. per 100 cc.; the hemoglobin 8.4 gm. per 100 cc. Again the serum iron was low, but the serum copper and free erythrocyte protoporphyrin were now elevated. She was taking food well and was less irritable. A nine-day mineral and nitrogen balance study was performed at this time and was tolerated well. Total blood lipids were normal, and the protein-bound-iodine level was found to be 3.6γ at this time.

On Oct. 11, 1955, at 7 months of age, C^{14}-labelled phenylalanine was given intravenously, and periodic blood samples were taken to measure the rates of synthesis of plasma protein and of protein loss from the plasma.

She was discharged from the hospital on the 35th day. Edema was not evident, there had been some weight gain, and the plasma total protein level was 4.7 gm. per 100 cc. The hemoglobin was 11.9 gm. per 100 cc., and her weight had increased to 7220 gm.

The patient was seen at weekly intervals in the outpatient clinic. For the first time she was not irritable, and gained weight steadily in the absence of edema. At 8 months of age the total proteins in the serum were 5.7 gm. per 100 cc., the hemoglobin 12.0 gm. per 100 cc., and the body weight was 8280 gm. The liver edge was no longer palpable.

In the following three months, the patient had minor respiratory-tract infections without complication or sequellae. At 11½ months of age, she weighed 9780 gm. and was 72 cm. in length. She is no longer irritable; there is no hepatomegaly or edema, and her motor development is normal.

Case 4.—This Caucasian boy of Jewish ancestry was born on June 14, 1955, the product of an uneventful pregnancy and delivery. At 5 days of age many watery stools were passed, and the formula was changed from simulated human milk (Similac) to an evaporated milk, water, and a cabohydrate (Dextri-Maltose) mixture. Undue irritability and "fussiness" were first noted at about this time. He was seen by a physician at one month of age. His length was 21½ in., and his weight was 4120 (9 lb. 1 oz.). He was somewhat irritable and another formula change to homogenized cow's milk was made. A multiple vitamin supplement was prescribed. Within 24 hours an erythematous papular rash was noted on the face, and later appeared on the flexor surfaces of the wrists and elbows. A soy-bean milk substitute was tried but was not well tolerated. When cow's milk was again taken, the skin lesions became more extensive. At 7 weeks of age a goat's milk formula was started. Only minimum change resulted in the extent and nature of the rash over the next eight-week period. During this time, he was unduly irritable and cried much of the time. At 15 weeks of age, the mother noted periorbital swelling for the first time. A physician was consulted who suggested another trial of homogenized cow's milk. This affected the rash adversely and was discontinued. This formula change did not influence the facial swelling. The hemoglobin level determined at 15 weeks of age was reported to be normal.

From 15 to 20 weeks of age, the facial swelling increased, irritability and crying became even more constant, and the erythematous rash spread. During this time he was taking 37 oz. of goat's milk and generous servings of lamb, cereal, apple sauce, and pears daily. At 16 weeks of age he weighed 6900 gm. (15 lb. 3 oz.) and was 63.0 cm. (25 in.) in length.

When first seen by us at 21 weeks of age (4½ months) he was admitted to the hospital for study. His weight was 7400 gm. (16 lb. 4 oz.). The head circumference was 42.0 cm., the chest circumference 41.0 cm. The temperature and pulse and respiratory rates were normal.

The infant was markedly irritable and displayed an almost constant distressed cry. Abnormal physical findings were limited to the papular erythematous rash involving much of the face, neck, and upper extremities. Generalized edema was most noticeable about the eyes. The liver edge was palpable 2.0 cm. below the right costal margin.

The family history revealed allergic rhinitis, bronchial asthma, and frequent urticaria in members of both the mother's and father's families. The patient had one normal male sibling 2½ years of age. One sibling had died after surgical correction of a congenital heart lesion at 8 months of age.

During the first 24 hours in the hospital, laboratory studies revealed that the serum protein level was 3.2 mg. per 100 cc., with 2.2 gm. albumin per 100 cc. and 1.0 gm. globulin per 100 cc. A uniform reduction in all globulin components was demonstrated by Tiselius electrophoresis. The hemoglobin level was 10.0 gm. per 100 cc., and low serum iron and copper levels were found. The free erythrocyte protoporphyrin level was elevated. The total leucocyte count was 17,500/cu. mm. with 45% lymphocytes. The urinalysis, serum cholesterol, prothrombin time, serum electrolytes, cephalin flocculation, thymol turbidity, and cholinesterase activity were found to be normal. An aspiration biopsy of the bone marrow revealed normal cellular constituents without normoblastic hyperplasia. X-ray studies of the entire skeleton were normal.

The patient was fed a goat's milk formula, with lamb, rice cereal, pears, and apple sauce. He was irritable but ate well. During the 10 days in the hospital, his weight remained constant.

On each of the fifth, seventh, and ninth hospital

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days, 12.5 gm. of salt-poor albumin were injected intravenously (total 37.5 gm.). The serum albumin level rose from 2.4 gm. per 100 cc. to 4.1 gm. per 100 cc. without significant change in the globulin concentration, and without detectable loss of weight. The visible edema, however, diminished after the administration of the protein.

The formula was changed to Lanolac on the seventh hospital day, and this was taken well. This formula was continued after discharge from the hospital on the 10th day.

The patient was seen at weekly intervals after leaving the hospital. One week after discharge, the serum albumin had fallen to 3.3 gm. per 100 cc. and the total serum proteins were 4.3 gm. per 100 cc. Three weeks after leaving the hospital the albumin level was 2.8 gm. per 100 cc., and some edema about the face was noted. The hemoglobin was 7.9 gm. per 100 cc. A subcutaneous injection of 1.0 mg. of pyridoxine was given daily for three days and did not alter the clinical or laboratory findings.

In a two-month period after discharge from the hospital, there was essentially no change in the clinical status of the patient. He slowly gained weight and grew but 1.0 cm. in length. The total serum protein level remained at approximately 4.3 gm. per 100 cc. with 3.2 gm. per 100 cc. albumin and 1.1 gm. per 100 cc. globulin. The hemoglobin level remained at 7.0 gm. per 100 cc.

The patient was placed on medicinal iron (Fer-In-Sol, 0.6 cc. q. i. d.) eight weeks after hospitalization. A reticulocyte response to 7.3% was noted on the fifth day, and the hemoglobin rose to levels of 11.0 gm. per 100 cc. within three weeks. Iron therapy was discontinued after six weeks, and the hemoglobin levels have remained essentially unchanged since that time.

In the two-month period following hospitalization, the total serum protein levels rose to levels between 5.1 to 5.3 gm. per 100 cc. where they have remained to date.

Comment on the Clinical Findings

Two of the patients were boys, and two, girls. Each was born after an uncomplicated pregnancy at a normal birth weight. The birth order was variable. One patient (Case 3) experienced a severe illness during the second week of life. A second patient developed skin allergy in the second month of life (Case 4). The other two infants were well until the onset of the irritability and edema. Marked irritability and some mild gastrointestinal disturbances were the earliest symptoms in the course of each infant. Edema in the periorbital region heralded the onset of recognizable disease in all patients. Because of these symptoms, three of the children had numerous changes of formula, none of which improved the symptomatology more than temporarily. In no patient were the digestive symptoms severe or prolonged to the point where impaired nutritional status should have been a concern. Linear growth continued close to previously established rates. By the time edema became evident, each child was well established on a diet of pureed infant foods in addition to the milk formula. Each child had received dietary iron and complementary protein from pureed meats. All had received multiple vitamin drops daily from early infancy. Food intake and retention was commensurate with age in each child. Pallor was evident as soon as edema appeared. In three patients this pallor was associated with low levels of circulating hemoglobin concentration at the time these children were first seen. In the fourth patient, after normal hemoglobin levels, anemia developed during the period of study. The edema in each child was of such degree that the nephrotic syndrome was suspected at the initial clinic visit. Proteinuria was never found, in spite of repeated attempts to demonstrate protein in the urine of all the infants.

Each patient was hospitalized shortly after the first clinic visit because of the irritability, gastrointestinal symptoms, and pallor. In general, a reduction of marked and proportional degree of plasma albumin and all globulin fractions was revealed in studies of the plasma proteins. By the usual hospital tests, no insight was gained into the mechanism responsible for these reductions. Intestinal malabsorption, hepatic and renal dysfunction, and caloric deprivation could not be demonstrated. In three patients moderate degrees of hypochromic anemia were present at the time of first observation during the illness. In the fourth the anemia appeared slowly during the first three weeks of observation. In all patients the anemia

became severer as time passed but did not appear to be responsible for any significant symptoms.

Signs of recovery appeared shortly after admission to the hospital in the first patient observed, who was also the oldest at the time of onset (Case 1). Weight loss accompanied by rising serum proteins and disappearance of irritability occurred during the first week of hospitalization in this child. Recovery was evident in all patients between the seventh and ninth month of life. The patients (Cases 1 and 2) whose onset of disease appeared at age 7 months and 6 months, respectively, displayed the shortest intervals between onset of symptoms and time of recovery. The patients in cases 2 and 3 recovered at approximately the same age, but, commensurate with the earliest onset, they displayed a more protracted period of symptoms.

The appearance of hypoproteinemia and anemia as a result of inadequate or improper diet seemed unlikely in these infants, whose linear growth rates were normal when symptoms first appeared. Evaluations of measurements of body weight were always in doubt because of the presence of edema; nutritional status could not be estimated from this measurement. The degree of digestive upset described by the parents did not seem to justify a diagnosis of malnutrition due to decreased intake, vomiting, or intestinal malabsorption. A meticulously detailed dietary history was obtained from each child's parents in an attempt to find a single brand of prepared infant food or formula common to the entire group. This attempt failed. It was felt, however, that a period of observation of the infant's feeding habits under more objective conditions than seemed possible in the home was necessary to confirm this point. In the hospital each child was fed the same milk formula and the same diet of pureed foods that he had received at home. In general, the same feeding schedule was maintained. The amounts of food retained were accurately recorded. The intakes of these children in terms of calories, protein, minerals, vitamins, and variety of food during the period of hospital observation were considered within the limits of normal when calculated according to tables of food values. Although it was obvious that the digestive upsets were real, no vomiting or diarrhea of more than a mild degree was observed.

Each baby was very irritable during the first several hospital days, but this symptom promptly improved greatly or disappeared entirely without specific therapy. The degree of irritability did not seem to be related to the severity of the hypoproteinemia or anemia, or to the amount of indigestion present.

Anemia was present when three of the four patients were first seen with complaints of edema, irritability, and pallor. The first three infants were 1, 4, and 7 months of age, respectively. The fourth infant was 4½ months of age when seen with these complaints, and the hemoglobin level was only minimally reduced at the time of his initial examination. Anemia of severe degree developed rather rapidly during the subsequent period of observation. The onset of swelling, irritability, and pallor was insidious. Except in the fourth patient (Case 4) there was no way of determining what the time relationship might have been regarding the development of anemia, hypoproteinemia, and the primary symptoms.

The most prominent clinical finding related to the anemia was the presence of extreme pallor. The pallor was due in part to the striking edema present in each patient, and could not be well correlated with the hemoglobin level alone.

Irritability was a prominent part of clinical findings in these four patients and is a common finding in infants with anemia due to any cause, particularly when the anemia results from iron depletion. The degree of irritability in these patients was more marked than seen in other anemic infants. It did not appear to be influenced by the hemoglobin level. The patient in Case 4 demonstrated marked irritability for

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several weeks prior to any evidence of anemia.

A notable finding in each of the four patients was the persistence of a vigorous appetite in the presence of marked irritability. In each instance the infant had enthusiastically taken an optimal diet prior to the onset of symptoms and continued to do so during the course of his disease. Iron supplemented cereals, pureed fruits, vegetables, and meats were taken well in addition to milk. It was impossible to find any aspect of the dietary histories which would indicate that any one of the patients failed to ingest amounts of the nutrients required for hematopoiesis which are normally adequate at this age. That the ingested nutrients could not be assimilated because of some gastrointestinal disorder was not suggested by the clinical findings.

There was no evidence from clinical findings that the anemia was the result of excessive destruction of erythrocytes or loss of blood. Icterus was never observed. The color of the urine and stools was never suggestive of increased excretion of bile pigments or loss of hemoglobin. Slight enlargement of the spleen was observed in one patient on one examination. At other times and in the other patients, the spleen could not be palpated. The histories did not reveal any evidences of gross blood loss. Delivery of the mother had been uncomplicated in each instance, with nothing to suggest infant blood loss at the time of birth. None of the patients had been born prematurely.

Clinical evidences of bone-marrow failure other than inadequate production of hemoglobin were lacking. Undue susceptibility to pyogenic infection, which might suggest agranulocytosis, or small-vessel bleeding suggesting thrombocytopenia were never observed.

These preliminary observations suggested some internal derangement in protein metabolism was present in these infants. Extensive laboratory studies were undertaken in an attempt to define the abnormality more completely.

Laboratory Studies and Results

Liver Function.—Since the liver is the principal site of plasma protein formation, attempts were made to demonstrate alterations of other of its functions which would implicate abnormal metabolism of hepatic cells. Clinical evidences of liver disease, such as jaundice, acholic stools, and massive hepatosplenomegaly, were uniformly absent. Each patient, however, had a palpably enlarged liver after the onset of symptoms. The enlargement persisted during the active phase of the disease, and disappeared after recovery. It is possible that this enlargement represented fluid accumulation within this organ. Hepatic tenderness was not demonstrated. Several minor indications of abnormal liver function were demonstrated in the group and are summarized in Table 1. In no single case could these tests be interpreted as indicative of definite evidence of a significant clinical hepatic disorder, al-

TABLE 1.—*Summary of Liver Function During Acute Phase of Illness*

Case	pH Serum Cholinesterase	Thymol Turb. Units	Zn Turb. Units	Ceph-Chol. Flocculation	BSP Retention	Total Cholest. (Mg/100 Ml.)	Cholest. Esters (Mg/100 Ml.)	Prothrombin (%) Activity	Bilirubin Mg/100 Ml.)	Total Lipid (Mg/100 Ml.)
1	0.28	0.5	...	0(24 hr.) 0(48 hr.)	...	...	...	...	0	...
4	0.45	1.9 1.0	...	1+(24 hr.)	...	223 150	109 88	82	...	...
2	...	...	...	0(24 hr.) 0(48 hr.)	...	90 112	...	100	...	...
3	0.27	...	1	...	0 @ 60'	724	144	...	0.33 0.67	450

though in Case 2 the number of studies performed was inadequate. Seroflocculation tests are known to be dependent on protein constituents of the plasma. Despite the lowering of all plasma protein fractions, significant alterations of these tests were not found.

Visible lipemia was apparent in the sera of two patients. Cholesterol concentrations in these serum specimens were normal or slightly elevated for infancy. Total lipid concentration was normal in one of these specimens. The lipemic appearance of the serum was thought to be the result of an altered fat to protein ratio, due to the hypoproteinemia.

Two of three patients studied were found to have abnormally low activities of serum cholinesterase during the hypoproteinemic phase of their disease. Since this test has been shown to correlate well with general hepatic function, and more particularly with protein anabolism, it is tempting to speculate that this is evidence that protein production by the liver was impaired. Bleisch and Shwachman state that in anemia the serum cholinesterase values may be low in children with no evidence of liver disorder.[6] The hemoglobin levels of Case 1 and Case 3 were 7.4 gm. and 4.6 gm., respectively, at the time these low cholinesterase values were found. Hemoglobin in Case 4 was 10.0 gm. at the time the normal level of cholinesterase activity was found, although the plasma proteins were low. Unfortunately, no test was preformed on this patient at the time his hemoglobin level was low. Case 3 showed a return of this test to normal levels (0.5ΔpH units) after her recovery from the anemia, but at a time when the total serum protein levels were lowest (2.3 gm. per 100 cc.). One year after recovery from the acute phase of the illness, and at a time when no other disease was evident, Case 1 demonstrated a markedly elevated level of serum cholinesterase. The significance of this latter finding awaits further clarification.

Although minor evidence of liver dysfunction was demonstrated in this group of infants, it is not remarkable considering the degree of hypoproteinemia present. These findings do not eliminate the possibility that some primary hepatic abnormality is responsible for this disorder. In McQuarrie's original description of idiopathic hypoproteinemia, the patient was a young child with no clinical signs of liver disease other than the low level of serum proteins.[7] Postmortem examination, however, revealed widespread evidence of noninflammatory liver atrophy.[8] The spontaneous and complete recovery of the four patients reported here suggest that liver atrophy was not present, but a more reversible stage of hepatic cellular disease could have been primarily responsible for their disease. Liver biopsies were not performed, and thus the hepatic histology remains unknown at the present time.

Adrenal Cortical Function.—An excessive action of an adrenal steroid such as 17-hydroxycorticosterone is known to result in excessive gluconeogenesis from protein and high levels of urinary nitrogen excretion. An attempt was made to evaluate activity of the adrenal cortex in three of the patients.† Case 3 excreted normal levels of urinary 17-hydroxycorticosteroids during the balance period reported elsewhere in this paper. In addition, at another time, the excretion of these compounds was determined after the intramuscular administration of 25 I. U. of adrenocorticotropic hormone. A three-fold increase in the 24-hour excretion of 17-hydroxycorticosteroids resulted. This finding would indicate normal responsiveness of the adrenal cortex. Although the blood contained only 2.78γ per 100 cc. of 17-hydroxylated steroids when no exogenous adrenal stimulation was present, a normal response of the blood steroid level to the injection of adrenocorticotropin could be demonstrated.[9] Four

† The urinary 17-hydroxycorticosteroids were determined by the method of Reddy.[10] Blood 17-OH corticosteroids were determined by the method of Nelson and Samuels.[11] Urinary 17-ketosteroid excretion was measured by the method of Drekter and others.[12]

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hours after injection of 25 I. U. corticotropin (ACTH) intramuscularly, the blood 17-hydroxycorticosteroid level rose to 47.7 micrograms per 100 cc. The patients in Cases 1 and 2 also excreted normal amounts of urinary 17-hydroxycorticoids during their periods of hypoproteinemia. No tests of response to adrenal stimulation were performed on these patients, and the evaluation of adrenal function in these two children cannot be considered complete.

Urinary 17-ketosteroid levels were normal for the infancy period in these three patients. The results of the steroid studies are summarized in Table 2. It is our feeling that adrenocortical hyperfunction could not be implicated in the etiology of this syndrome.

TABLE 2.—*Urinary Steroid Excretion During Hypoproteinemia*

	Date (1954)	17-OH Corticosteroids (Mg. per 24 Hr)	17-Ketosteroids (Mg. per 24 Hr)
Case 3	9-19	2.5	0.3
	9-20	2.4	0.4
	9-21	2.1	0.3
	9-22	2.0	0
	9-23	2.1	0.35
	9-24	2.3	0.23
	9-25	1.8	0.25
	9-26	2.3	0-23
Case 2	11-26	0.96	0.18
	11-27	1.44	0.17
	11-28	0.74	0.39
Case 1	7-24	1.80	0.23

Protein Fractionations.—In all patients the total serum proteins were low, as measured by standard clinical laboratory techniques.[13] In addition, chemical fractionation methods demonstrated that both the albumin and total globulin fractions were depressed. In all patients the plasma albumin/globulin ratio remained normal. The actual values for each patient are tabulated in Table 3.

TABLE 3.—*Studies of Plasma Protein Concentrations*

	Date	TP	Albumin	Globulin	A/G
Case 1	7- 8-54	3.0	1.9	1.1	1.7
	7-13-54	2.8	2.1	0.7	3.0
	7-14-54	3.5	1.8	1.70	1.05
	7-24-54	4.6	3.2	1.4	2.3
	8- 7-54	4.8	3.7	1.1	3.4
	9- 9-54	6.6	4.0	2.6	1.5
	9-27-54	7.5	4.4	3.1	1.4
	10- 1-54	7.0	3.6	3.4	1.05
Case 3	2-16-55	7.9	4.3	3.6	1.2
	3-26-55	6.4	3.4	3.0	1.1
	2-17-55	6.4	3.2	3.2	1.0
	10- 5-55	6.1	4.1	2.0	2.0
	7-15-55	2.7	1.3	1.4	0.9
	7-20-55	3.3	2.3	1.0	2.3
	7-26-55	3.2	2.5	0.7	3.6
	8- 2-55	3.4	2.3	1.1	2.1
	8- 9-55	3.8	2.0	1.8	1.1
	8-16-55	3.4	2.4	1.0	2.4
	8-24-55	3.0	2.0	1.0	2.0
	9-12-55	2.3	1.7	0.6	2.8
	9-19-55	2.8	1.9	0.9	2.1
	10-25-55	4.7	3.2	1.5	2.1
	11-22-55	5.7	3.6	2.1	1.7
Case 4	10-17-55	3.1	2.1	1.0	2.1
	10-26-55	3.2	2.2	1.0	2.2
	10-29-55	3.6	2.4	1.2	2.0
	10-29-55		(Albumin 12.5 gm. IV)		
	10-30-55	4.2	3.0	1.2	2.5
	10-31-55		(Albumin 12.5 gm. IV)		
	11- 1-55	4.7	3.6	1.1	3.3
	11- 2-55		(Albumin 12.5 gm. IV)		
	11- 3-55	5.3	4.1	1.2	3.4
	11- 7-55	4.6	3.3	1.3	2.5
	11-10-55	4.2	2.8	1.4	2.0
	11-15-55	4.3	3.1	1.2	2.6
	11-21-55	4.1	2.7	1.4	1.9
	11-26-55	4.2	3.0	1.2	2.5
	12-20-55	4.8	2.8	2.0	1.4
	1-17-56	5.0	3.2	1.8	1.8
Case 2	11-24-54	2.6	1.7	0.9	1.8
	12- 1-54	3.6	3.1	0.5	6.2
	12- 6-54	4.3	3.7	0.39	9.4
	12-18-54	5.0	3.85	1.15	3.3
	1- 2-55	6.35	4.05	2.3	1.5

Electrophoretic analyses of the plasma proteins demonstrated a remarkable similarity of the protein patterns in each infant. The abnormalities of each patient studied in the active phase of the process included depression of levels of albumin, alpha$_1$ and alpha$_2$ globulins and gamma globulins. Albumin and globulin fractions appeared to be present in approximately one-third of normal concentration. The plasma protein pattern as determined by Tiselius electrophoresis in Case 1 is illustrated in Figure

Fig. 1a.—Electrophoretic pattern. Tracing of Tiselius electrophoresis of plasma of patient in Case 1 while hypoproteinemic. Albumin and all globulin fractions are depressed. Fibrinogen content is normal.

Fig. 1b.—Electrophoretic pattern. This specimen was taken two months after the specimen shown in Figure 1a. The electrophoretic pattern at this time is entirely normal.

1a and is typical of all patients. Figure 1b demonstrates the return to a completely normal pattern two months later. It was of interest to discover in these patterns that little or no gamma globulin was detectable. There had been no clinical evidence to suggest that any of these infants had experienced an unusual susceptibility to infection or demonstrated any inability to recover from the minor infections they had experienced. Further consideration of this point suggests that the depression of gamma globulin concentration is merely proportional to similar depressions of other protein fractions. Levels of gamma globulin below approximately 100 mg. per 100 cc. are not usually detected by either paper or free electrophoresis.[14] In addition to the Tiselius fractionation shown in Figures 1a and 1b, paper-strip electrophoresis was performed and yielded similar findings. In contrast to the lowered values of the albumin and globulin fractions, it is of interest that the fibrinogen content was not regularly depressed to this same extent. No immediate explanation for this finding is available, and it appears that either fibrinogen turnover has not been affected or that compensation of either the production or the destruction rate has occurred.

Immunity.—The recognized importance of the gamma globulin fraction of the plasma as a factor in the normal immune mechanism prompted investigation of this aspect of the problem in the infants under discussion. All patients showed a virtual absence of gamma globulin when tested with either paper or Tiselius electrophoresis. The normal infant shows decreasing concentrations of this antibody-containing plasma protein fraction from birth until the third month of life.[15] After the third month, the concentration of this plasma protein fraction as well as others, increases at a steady rate. Recent evidence suggests the normal infant produces little of its own serum protein early in life, and this may be the reason for the normally decreasing serum protein concentrations.‡ Orlandini and others recently reported that the concentration of circulating gamma globulin in 13 infants age 3 to 6 months was 0.39± 0.09 gm. per 100 ml., while total globulin levels were 2.15±0.21 gm/100 ml.[15] It is in the age group of two to three months that these concentrations are lowest in the normal infants. All infants in this study had passed this period of physiologic low concentration of plasma protein when signs of edema first appeared.

Although the gamma globulin levels have not been measured chemically in our patients, the virtual absence of it in electrophoretic patterns indicates that less than 0.1 gm/100 ml. was present. The gamma fraction in Orlandini's series represented

‡ References 16 and 17.

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approximately 5%-8% of the total protein content and roughly 14%-22% of the total globulin fraction. Since more sensitive direct chemical measurements of gamma globulin were not available to us, it was not surprising that gamma globulin was not detected at the low levels of total protein and globulin exhibited by our patients. For this reason, an attempt to find specific antibodies was made, taking advantage of the more sensitive immunologic techniques. With these methods it was possible to demonstrate that certain immune bodies which are usually in the gamma fraction, were present in each patient. A summary of the immunologic tests performed on our patients is given in Table 4.

TABLE 4.—*Immunology*

	Case 1*	Case 3*	Case 4
Schick Test	9-17-54 Negative	7-19-55 Negative 9- 1-55 Positive 11-28-55 Negative	Neg.
Antibodies To: (Blood Type)	O	A	
AB Cells	Agglutinates A & B cells	Anti-A Negative Anti-B 1:8	
Diphtheria	0.1 U/ml.	9- 1 0 U/ml. 9-27 0.009 11-28 0.03 U/ml.	
Tetanus	0.1 U/ml.	9- 1 0 9-27 0.3 U/ml. 11-28 1.0 U/ml.	
Pertussis	+	9- 1 1:32 9-27 1:16 11-28 1:16	
Vaccination	4-20-54 I° Immune reaction		
Typhoid		9- 1 1:8 9-27 1:8 11-28 1:8	

* These studies of antibodies to diphtheria, tetanus, pertussis, and typhoid were made by the Cutter Laboratories, Berkeley, Calif., courtesy of Dr. E. B. McLean.

Two of the patients in this series were tested for blood-grouping antibodies in the serum. Each showed normal ability to agglutinate red blood cells of known major blood groups. One patient (Case 1), whose major blood group was O, demonstrated serum antibodies against type A and B red blood cells. The patient in Case 3 was determined to be blood group A; antibodies against type B cells were present in a dilution of 1:8.

Schick tests were applied to three patients. Case 1 had been given three injections of diphtheria, pertussis, and tetanus antigen in a customary fashion several weeks before the illness began. The child was Schick tested after the electrophoretic pattern had returned to normal, approximately two months after the height of her illness. No response was elicited from the skin test. Serum levels of antitoxin at this time showed protective levels against diphtheria and tetanus. Pertussis antibodies were also present. No antigenic stimulation was administered between the hypoproteinemic period and the time of testing. This does not indicate that protective levels of antibody were present during the hypoproteinemia, but demonstrates that as new protein was formed, the ability to continue producing antibody from an earlier stimulus was retained.

Nine months after recovery from the

hypoproteinemia, this child developed fulminating meningococcemia. In spite of a clinical picture of profound shock and septic necrosis of large areas of skin, she recovered in a short time with the aid of antibiotic and steroid therapy. Electrophoretic analysis of the plasma proteins at the onset of the sepsis revealed normal levels of all components including gamma globulin.

The patient in Case 3 had received no immunization at the time of her first Schick test. There was no reaction to this test, indicating clinical immunity to diphtheria. This is not unexpected, since she was only 3 months old at this time. One month later, however, a second test resulted in a positive skin reaction, suggesting she had lost her passively acquired immunity. Serum antibodies against diphtheria and tetanus could not be demonstrated at this time. An injection of diphtheria-tetanus fluid toxoid was then given. Two weeks after this injection, a small amount of diphtheria antitoxin and a moderate level of tetanus antitoxin were detected in the serum. After a second injection of this combined antigen, the Schick test again became negative. During the period of these observations, several electrophoretic patterns demonstrated that no gamma globulin was detectable by this method. The total serum protein level was falling during this time when the child was forming antibodies. This observation suggests that the protein defect in this infant was associated with a rapid degradation of serum immune proteins, rather than with a decreased rate of serum immune protein formation.

The patient in Case 1 was successfully vaccinated against smallpox before the onset of hypoproteinemia. Several attempts to revaccinate this child after edema had subsided after recovery from her illness were unsuccessful for unknown reasons, but eventually an immune reaction was observed. Studies by others have shown that patients developing complications to vaccination often have low serum levels of gamma globulin.[18] It was therefore felt unwise to vaccinate any of these children during the period of hypoproteinemia.

These studies do not justify any broad conclusions concerning the relationship of immunity to the concentration of circulating total gamma globulin and other globulin fractions known to contain immunologic activity. The virtual absence of gamma globulin from the serum as demonstrated by electrophoretic analysis was not accompanied by a loss of immunity in these infants. In addition to the laboratory demonstrations of immune bodies, the clinical observation was made that these patients were relatively free of infection. Two of the patients had older siblings that may have provided exposure to infection in their homes. All patients were hospitalized for periods up to two months and were protected by the usual infant-ward precautions against cross infection. Although respiratory-tract infection was observed, neither prolonged nor severe reactions to it were present. The female infants were subjected to catheterization for quantitative collection of urine during metabolic studies of several days duration. Bacterial infection of the urinary tract, commonly seen under these circumstances in normal infants, did not result. A diagnosis of congenital agammaglobulinemia was not made.

Over-All Nitrogen Balance.—The diagnostic procedures used in the initial study of the patients failed to identify a basic abnormality responsible for these patients' clinical findings. Renal or hepatic disease, in the usual sense, had been excluded as a cause of their disease. The over-all nutritional state of these infants was good, and the wasting and growth failure usually seen before nutritional hypoproteinemia occurs was absent. The stools did not appear either large or foul. Steatorrhea and azorrhea were not evident clinically. To support or refute these clinical findings, it was felt that a precise investigation of nitrogen absorption and utilization would be essential.

The nitrogen absorption and utilization data were obtained by metabolic balance

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studies on the two girls. Quantitative analyses were made of the dietary intake and the stool and urinary excretion of nitrogen, potassium, and phosphorus.

During the study periods, the infants rested on specially constructed frames. Indwelling catheter insured quantitative collection of all urine. Carmine or charcoal was fed at the beginning and end of each study period to aid identification of stool specimens by periods. Each infant was fed a liquid milk preparation only. The milk preparation was adjusted in content in order to supply adequate caloric and nitrogen intake in that amount of formula each child would take in "demand" feedings (Table 5). A preliminary period of one week was allowed under conditions identical to the test period. Collections were begun only after it was apparent that the test diet was well tolerated, and a reasonably stable metabolic state had been attained on the diet. The diets and the urine and stool specimens were analyzed in the laboratory for their content of nitrogen, phosphorus, and potassium.§ Fat content was measured in one of the studies. Caloric values were determined by calculation using standard caloric value charts.

TABLE 5.—*Composition of Diets for Balance Studies*

Pt. Case	Body Wt. (Kg.)	% Composition			Calories Derived from			Average Calories Kg/Day*	Nitrogen* Mg/Kg/Day (Av.)
		Carbohydrate	Protein	Fat	Carbohydrate	Protein	Fat		
1	8.6	5.32	3.66	3.56	32%	22%	46%	84	74.5
3	7.8	4.7	3.96	3.70	49%	23%	28%	117	76.4

Results of Balance Studies

CASE 1.—Almost immediately on admission to the hospital for studies, diuresis, weight loss, and rise of serum proteins began. Since this was the first of the four patients studied, the interpretation of these findings as signs of recovery was made only in retrospect. As will be seen in Figure 2, there is no question that the balance study was performed on this child early in the recovery phase.

Simultaneously with the last two days of the three-day balance period, 150 mg. of N^{15}-labelled glycine was fed. Results of this isotope study are discussed in detail, elsewhere.[19]

Table 6 describes in detail the oral intake and the urinary and fecal excretion of nitrogen, potassium, and phosphorus by this patient. Although there had been spontaneous and precipitous loss of edema fluid in the week preceding the study (Fig. 2), the weight remained constant over this three-day balance period. The caloric intake during the study was below a desirable level for an 8-month-old child. A total of 2240 calories was ingested during this three-day study. Since no attempt was made to limit the milk intake, the amount ingested was determined by the child's appetite. The protein intake, however, was judged to be adequate. The formula fed this infant was undiluted homogenized milk.

Since the protein intake of normal infants is derived largely from milk, it is not surprising that this discrepancy of caloric and protein intake exists here. Ordinarily, pureed foods which are relatively low in protein content supplement the caloric intake of the infant at this age. As stated previously, this type of mixed diet was the usual fare of this infant, but, because of the technical difficulties involved in the use of solid type food in balance-study measurements, it was decided to use milk as the only food during this study.

On each day of the study, the nitrogen balance remained positive; that is to say, the protein nitrogen intake was greater than

§ Nitrogen, potassium, and phosphorus were determined in the laboratory of Dr. William Adams.

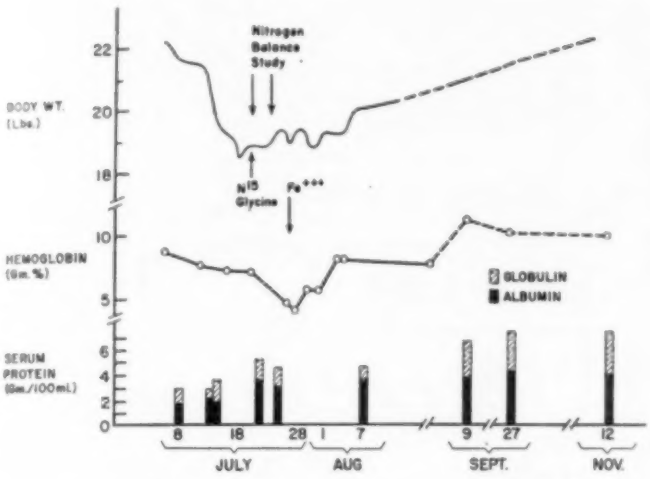

Fig. 2—Graphic representation of body weight, hemoglobin concentration, total and fractional protein values in Case 1.

the combined urinary nitrogen and the average fecal nitrogen (Table 6). On the first day, when the caloric intake was 110 calories per kilogram, the nitrogen intake was considerably greater than the output. The study is to be criticized because of the low caloric intake, but certain conclusions can be drawn in spite of this shortcoming. It would appear that the hypoproteinemia could not be the result of either deficient protein intake or over-all negative balance for any reason. The continuing normal rate of the child would tend to substantiate this conclusion.

It would appear from this study that the absorption of protein was normal, since only 4% of the ingested nitrogen appeared in the stool. In contrast to this, 21% of the ingested fat was present in the fecal content. The significance of this finding is not clear. The inefficient fat absorption adds further to the caloric inadequacy of the diet.

The retentions of potassium and phosphorus were measured simultaneously with those of nitrogen. Although the period of study was too short to justify critical evaluation, it would appear that these minerals were retained in approximately the same degree as would be expected for the formation of new tissue.[20]

Case 3.—The balance study performed on this child was at a time when moderately severe edema was present. Although an attempt to reduce the edema had been made by providing an intake of sodium lower than that of undiluted cow's milk, the puffiness remained (Fig. 3). The patient was fed a formula which contained only 14.0 mEq. of sodium per liter. During the study the intake of sodium varied from 1.8 to 2.4 mEq/kg. of body weight, and averaged 2.1 mEq/kg. Since the patient had taken this low-sodium diet for a two-week period preceding the balance measurements, it was felt that the necessary adjustments to it had become stabilized. The total intake of formula was determined by the child's appetite. A moderate variation of volume of intake occurred from day to day, but the caloric intake was always above 100 calories per kilogram of body weight. The calculated caloric intake varied from 104 to 133 calories per kilogram, and averaged 117 calories per kilogram per day. Approximately 23% of these calories was derived from cow's milk protein, insuring a liberal protein intake (3-4 gm/kg. of body weight per day). The carbohydrate and fat were present in those proportions found in cow's milk (Table 5). As in the preceding study, all food was offered in a liquid form, and the usual vitamin supplements were added. (Details of the balance study results on

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TABLE 6.—*Balance Summary (Case 1)*

Day	Intake N (Gm.)	Urine N (Gm.)	Stool N (Gm.)	Balance N (Gm.)	Intake K (mEq.)	Urine K (mEq.)	Stool K (mEq.)	Balance K (mEq.)	Intake P (Gm.)	Urine P (Gm.)	Stool P (Gm.)	Balance P (Gm.)
1	8.62	4.119	0.339	+4.162	55.90	34.62	0.323	+20.957	1.260	0.641	0.190	+0.429
2	5.20	4.443	0.306	+0.451	34.70	35.09	0.476	−0.866	0.770	0.648	0.212	−0.090
3	5.40	4.547	0.306	+0.547	34.00	34.90	0.476	−1.376	0.800	0.707	0.212	−0.119
Total	19.22	13.109	0.951	+5.160	124.6	104.61	1.275	+18.72	2.830	1.996	0.614	+0.220
Average Daily	6.41	4.369	0.317	+1.720	41.53	34.87	0.425	+6.240	0.943	0.665	0.204	
Output Total Intake×100		68.1	4.9	73.1								

TABLE 7.—*Balance Summary (Case 3)*

Day	Nitrogen				Potassium				Phosphorous			
	Intake (Gm.)	Urine (Gm.)	Stool (Gm.)	Balance (Gm.)	Intake (mEq.)	Urine (mEq.)	Stool (mEq.)	Balance (mEq.)	Intake (Gm.)	Urine (Gm.)	Stool (Gm.)	Balance (Gm.)
1	5.73	3.66	0.852	+1.21	30.41	16.9	4.975	+8.54	0.972	0.413	0.324	+0.235
2	5.78	3.67	0.862	+1.25	31.10	18.0	4.975	+8.12	0.973	0.438	0.324	+0.211
3	5.13	2.62	0.862	+1.65	26.81	16.0	4.975	+5.84	0.786	0.295	0.324	+0.167
4	4.74	2.67	0.862	+1.21	28.99	17.4	4.975	+6.62	0.897	0.317	0.324	+0.256
5	4.42	4.17	0.862	−0.61	26.17	22.6	4.975	−1.40	0.836	0.473	0.324	+0.039
6	5.41	4.48	0.862	+0.07	30.65	24.0	4.975	+1.68	0.984	0.499	0.324	+0.161
7	5.8	5.50	0.862	−0.56	34.29	30.0	4.975	−0.68	1.087	0.360	0.324	+0.203
8	5.79	3.13	0.862	+1.80	32.43	25.4	4.975	+2.05	1.007	0.490	0.324	+0.193
Total	42.8	29.90	6.896	+6.024	240.86	170.3	39.80	+30.76	7.542	3.485	2.592	+1.465
Average Daily	5.35	3.73	0.862	+0.75	30.11	21.28	4.975	3.84	0.942	0.435	0.324	+0.183
Output Total Intake×100		69.7	16.1*	85.8								

* Stool values high owing to contamination of stool with urine during collection.

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Fig. 3.—Case 3. Photo taken preceding balance study. Note generalized edema.

Case 3 are tabulated in Table 7.)

During the first four days of this study period, the weight increased 250 gm. The urinary output of water remained approximately 50% of the volume of fluid intake. In the last three days of the study, a loss of 155 gm. of body weight was accompanied by a diuresis of 75% to 80% of the daily ingested fluid volume. Unfortunately, a water and chloride balance study was not performed. It seems likely that these rapid changes in body weight were the result of spontaneous shifts in intercompartmental fluid balance, such as are often seen in patients with hypoproteinemia.

Although the net change in body weight over the eight-day balance period was a gain of 135 gm., one cannot assume that this is a true gain in body tissue. The fluctuation of water balance during the study must be considered. The total gain of 6.02 gm. of nitrogen during the balance period agrees remarkably well with this net gain in body weight. However, when Talbot's factor for fat-free and extracellular-fluid-free protoplasm is applied ($N \times 27 =$ Net Change of E. C. fluid-free protoplasm), one finds that $6.02 \times 27 = 162.34$ gm.[21] According to this theoretical calculation, a small net loss of body fluid may have occurred. A calculated increase in extracellular-free protoplasm of 162 gm. during a period when only 135 gm. of body weight was gained suggests a loss of edema fluid.

Urine collections from Case 3 were imperfect because of some leakage of urine around the catheter early in the study period. The metabolism frame was so constructed, however, that the urine was not lost; rather, it was collected in the stool container. The values reported on this patient for urinary content of nitrogen and minerals on days one through four are lower than the true values. The stool collection was pooled during the entire eight-day study period. Therefore, the total and average daily fecal content of nitrogen, potassium, phosphorus represents fecal excretion plus a small amount of urine accidentally included. This does not modify the final over-all balance figures, since urine and stool data are added together to give total output. It suggests that a fraction of the dietary nitrogen greater than that recorded was present in the urine during the study.

As would be expected in an unstable state of fluid equilibrium, the potassium balance was erratic. The excessive retentions of potassium recorded in the early part of the balance period may be the result of excessive perspiration which was noted during this study. Measured balances of potassium are falsely high when sweat losses are not considered. As the diuresis of water began, urinary potassium losses increased. It is also possible that the low level of dietary sodium influenced tubular mechanisms to favor potassium loss in the urine. Data is incomplete on these points, however.

The over-all balance of phosphorus remained positive throughout the study pe-

riod. With the exception of the first day of the diuresis, this retention seems proportional to the dietary intake of phosphorus.

Comment on Balance Studies

The studies of over-all nitrogen metabolism in these two infants demonstrate that the hypoproteinemia was not on the basis of a failure to retain dietary nitrogen. In one infant the caloric intake was judged adequate before the balance study began but was inadequate during the three days of measured intake. In spite of this, she was able to retain nitrogen. The caloric intake of the second child was slightly above the recommended level of 110 calories per kilogram of body weight.

Kaye has found normal infants usually retain approximately one-half of the ingested protein nitrogen at the level of intake our patients were receiving.[22] Throughout the study period, the percentage of ingested nitrogen appearing in the urine in Case 3 remained high. This finding could be interpreted as being compatible with a diversion of the dietary nitrogen to nonprotein pathways. Caloric underfeeding, ketosis, stress, and the administration of vitamin B_{12} in large amounts[14] are among the "antianabolic" forces which might be responsible for this diversion. None of these factors could be demonstrated in this patient. It would appear from other evidence to be presented subsequently that this rapid elimination of body nitrogen is a reflection of the fundamental defect in plasma protein catabolism in this disease and that amino-acid nitrogen of dietary origin is excreted in normal amounts.

The degree of total nitrogen retention is similar to or slightly less than that found in normal infants of comparable weight and nitrogen intake. Retention of both potassium and phosphorus was also demonstrated, likewise indicating that tissue formation and not destruction was predominating. Spontaneous shifts in water balance make more precise interpretation of this data unjustified. The findings, together with normal growth rates, favor the conclusion that the low serum protein level is not a result of over-all negative protein balance. Other than this, no conclusions as to mechanisms responsible for the hypoproteinemia can be drawn from these balance studies.

Rates of Plasma Protein Formation and Degradation.—The preceding studies provided indirect evidence that the circulating protein disturbance was the result of a more rapid than normal degradation of serum proteins. An attempt was made to obtain more direct evidence concerning this mechanism by studying the rates of turnover of isotope-labelled amino acids in three of the patients. The results of these studies are reported in detail in a subsequent paper.[19]

Two of the infants (Case 3 and Case 4) were studied using C^{14}-labelled phenylalanine. Both of these patients demonstrated approximately the same rate of incorporation of this essential amino acid into serum proteins as did two normal infants of comparable age. The rate of incorporation of this isotope into circulating albumin compared favorably with the results obtained by Kelley and co-workers in two older children.[23] Methionine labelled with S^{35} was used in the latter's study. In Case 1 the patient was fed N^{15}-labelled glycine at the beginning of her recovery period but at a time when the total protein level was low. This child demonstrated a somewhat greater rate of incorporation of isotope into plasma protein at this time than did the other infants. She was restudied at age 2 years, approximately 18 months after apparent clinical recovery. Phenylalanine labelled with C^{14} was used in this second study. At this time, when the total plasma protein level and electrophoretic pattern were normal, the isotope incorporation rate was similar to that found in the normal infants and the two hypoproteinemic infants studied by this method.

The rate of disappearance of C^{14} from the albumin and globulin fractions of the circulating protein was studied in two infants with hypoproteinemia and in two nor-

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mal infants of comparable age. The albumin fraction in the hypoproteinemic infants was found to have a rate of loss of isotope approximately twice that of the normal infants. The half lives of the isotope label in the infants with hypoproteinemia were approximately 15 and 12 days. The normal infants demonstrated half lives of approximately 29 and 22 days. The latter figures compare favorably with the results obtained in adults by other authors. When the patient in Case 1 was studied by this method at age 2 years, a half life for albumin was found to be approximately 23 days. Turnover rates of globulin were similarly increased in the hypoproteinemic infants.

The rate of urinary excretion of N^{15} was studied in Case 1 during hypoproteinemia. She excreted 31.9% of the dose of N^{15} administered as glycine N^{15} orally in the first 24-hour period. An additional 6.5% was excreted in the subsequent 24-hour period. Normal values have not been established for infants. Sprinson and Rittenberg found similar values to those reported here in their studies of adults.

These isotope studies suggest that there is no failure of plasma protein synthesis from dietary or injected amino acids compared with the normal infants to account for the low levels of circulating protein. The short-end half life of the isotope bound in the albumin and globulin fraction strongly suggests that hypoproteinemia is on this basis, primarily. Since, however, the concentration of circulating protein is dependent on both the rate of production and the rate of degradation, a relative slowness of the synthesis rate is apparent. This interpretation is also implied by the fact that the number of new amino acid moieties present per protein molecule in the decreased total amount of circulating protein is no greater than that found in the infants with normal protein levels. A greater total amount of protein is circulating in the normal infant, and therefore a greater total number of labelled amino acid molecules must be incorporated to result in the same concentration of isotope per molecule of protein. If reutilization or "feed back" of labelled amino acid from other body proteins plays a significant part in these studies, the rapid decay apparent here could result from a failure of utilization of labelled amino acids from these internal sources. Since labelled dietary amino acids were excreted in normal amounts during the 48-hour period following their ingestion, it would appear that their utilization is at a rate similar to that of a normal adult.

It would appear from these studies, therefore, that an imbalance between the rate of production and the rate of degradation is responsible for the low concentration of plasma proteins. Since normal values for each of these rates are not well established, it can only be stated that the infants with hypoproteinemia incorporated isotope-labelled amino acids into plasma protein at approximately the same rate as the normal infants did. The loss of the isotope label from this protein, however, proceeded at twice the rate in the hypoproteinemic infants that it did in the normal infants. In one patient who was studied 18 months after clinical recovery, the imbalance appeared to be gone. Her serum protein levels were normal. Normal rates of incorporation of phenylalanine 3-C^{14} into circulating protein and loss of isotope from the protein were found. Although it is conceivable that clinical recovery could have occurred in the face of continued rapid degradation of circulating protein, this was not the case. The transitory nature of this condition seems emphasized by this latter study.

Immunological Properties of Plasma Albumin.—The increased turnover rate of the albumin fraction of the plasma in the patients with hypoproteinemia could be either the result of a disturbance in the mechanisms responsible for the breakdown of protein, or a difference in the physicochemical structure of the molecule itself. No difference in electrophoretic mobility was noted in the albumin of the hypoproteinemic patients as compared with

normal persons. An attempt was made to demonstrate a difference in the antigenic properties of the albumin molecule by antigen-antibody reactions.

Method of the Study

Albumin was prepared from the plasma of normal adults, normal infants ranging in age from one day to eleven months, and two of the patients with hypoproteinemia (Case 3 and Case 4). The same methods of separation and lyophilization were used as were employed in the experiments concerning isotope incorporation.[39] Twenty milligrams of each albumin sample was dissolved in 0.5 cc. of sterile normal saline solution, and an emulsion prepared by the addition of an equivalent volume of Freund's adjuvant immediately before injection. This antigenic mixture was injected intramuscularly into adult male white rabbits. Three injections were given each animal at weekly intervals. Two rabbits were immunized with the albumin of each person. One week following the third injection, 20 mg. of the appropriate albumin dissolved in 0.1 cc. of sterile normal saline was injected intradermally into each rabbit. A violent local reaction of the skin surrounding the injection site resulted in the test animals, and no reaction was present in the control animals. Two weeks following the intradermal injection, each rabbit was bled. After complement inactivation, the rabbit serum was tested for the presence of precipitins by the ring test using albumin as the antigen. All sera from the immunized rabbits showed the presence of precipitins in antigen dilutions ranging from 1:100 to 1:51,200. No reaction was observed at any dilution in the sera of control rabbits that had received saline and adjuvant only.

Ouchterlony agar plate precipitin tests were then carried out according to the modification of Wilson and Pringle.[26] Antigen from the normal adults, normal infants, and hypoproteinemic infants was added to the agar plate wells so that the final concentration of albumin was 0.02 mg/ml. Rabbit serum was diluted 1:1 with agar and added to the agar plate wells. The plates were incubated at room temperature for four weeks, and were inspected every three days during this period, and the pattern of precipitation was recorded.

Results

Under the conditions described above, it was possible to demonstrate that a single continuous line of antibody-antigen precipitation formed in all combinations of testing the above-mentioned groups. Control tests were maintained throughout in the form of substitution of normal sterile saline solution for either the antigen or the antibody component of the test plates. In addition, sera from control animals failed to develop precipitin lines when tested against each subject's albumin. Of particular interest was the demonstration of a continuous single line of antigen antibody precipitation when the serum of rabbits immunized against albumin obtained from a normal newborn infant was tested against the albumin derived from the two patients with hypoproteinemia (Fig. 4). Single continuous lines were also demonstrated when the serum of rabbits immunized with albumin from each hypoproteinemic patient was tested against albumin from the other hypoproteinemic infant (Fig. 5). Similar reactions were observed when rabbit anti-hypoproteinemia-albumin serum was tested against both normal adults and normal infants.

It would appear that, under the conditions employed, the immunologic properties of the albumin from two of the patients with hypoproteinemia were the same as those of albumin from normal adults and normal infants. The dilutions of antigen used in this procedure were necessarily great, since the availability of albumin antigen from the infants with hypoproteinemia was limited. Although multiple antigens may be demonstrated with this technique under the proper conditions, only a single component was found in our system. One would conclude that the major antigenic component of the albumin of the two patients suffering from hypoproteinemia was identical with that of normal adults and normal infants.

Hematological Findings.—Laboratory studies and observations regarding the progressive alterations in circulating hemoglobin were somewhat complicated by the repeated venesections necessary for the detailed laboratory evaluation of each patient. Initial observations in each instance were adequate to define the morphological alterations in the peripheral blood and bone marrow before iatrogenic factors influenced the

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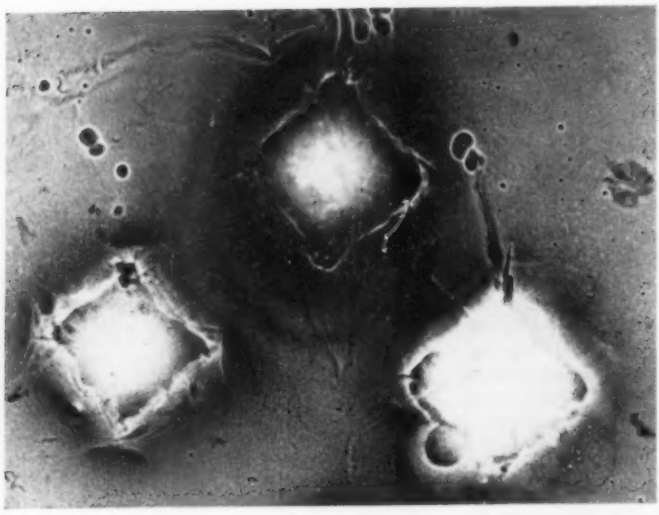

Fig. 4. — Ouchterlony agar plate precipitin test. The top well contains serum from a rabbit immunized by injecting albumin from a normal infant. The left hand well contains albumin from Case 4. The right hand well contains albumin from Case 3. Note continuous single V-shaped line of precipitation equidistant from each of the wells.

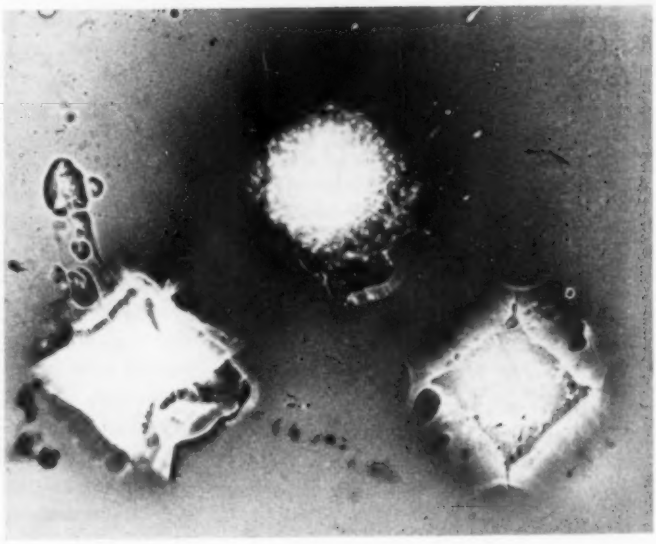

Fig. 5. — Ouchterlony agar plate precipitin test. The top well contains serum from a rabbit immunized against the patient in Case 4. The left hand well contains albumin from Case 4. The right hand well contains albumin from Case 3. A single, continuous, V-shaped line equidistant from each well is seen.

hematological findings. These findings are summarized in Table 8.

The erythrocytes in the peripheral blood smear were somewhat smaller than normal and poorly filled with hemoglobin. There was no unusual polychromatophilia, stippling, or presence of nucleated erythrocyte precursors in the peripheral blood. A persistent reticulocytopenia was uniformly present in all infants prior to the administration of iron. Even after considerable blood loss necessitated by the many laboratory studies, reticulocytosis was not found. The morphological alterations in the erythrocytes did not differ from those commonly encountered in iron deficiency states (Fig. 6). The moderate degree of microcytosis is clearly demonstrated in the Price-Jones curves done on an early blood film from three of the patients (Fig. 7). A

moderate degree lymphocytosis was observed in each patient, being most marked in Cases 1 and 3. The total leucocyte counts were consistently elevated (9000 to 25,000 per millimeter), with 45% to 82% of these cells being mature, small lymphocytes. The ratio of lymphocytes to granulocytes did not differ significantly whether the total leucocyte count was near normal or quite strikingly elevated.

Several platelet counts from each patient gave results that did not vary from our normal values.

Aspiration biopsies of the tibial marrow was available from each patient soon after they were first seen with symptoms. In each instance a highly cellular specimen was obtained without difficulty. Abnormalities could not be identified in either the megakaryocytes or in the granulocyte precursors. Although lymphocytes were present in abundance in the marrow aspirate in some instances, it is not possible to attach any significance to this finding in view of the lymphocytosis present in the peripheral blood which was obviously mixed with our aspiration specimen. Plasma cell counts were normal in each of the four patients.

The most striking finding in the marrow material was the absence of any morphological changes indicative of increased erythropoietic activity. Late normoblastic hyperplasia as seen in pediatric patients with iron, copper, and pyridoxine deficiency

TABLE 8.—*Peripheral Blood Counts*

Date (1954)	HGB Gm./100 Ml.	RBC M^3	Retic %	Plat M^3	WBC M^3	WBC Differential				
						PMN	Band	Eos.	Lymph.	Mono.
Case 1										
7-20	7.1	3.23	2.0		10,600	24		8	67	1
7-26	4.7	2.95	2.0							
7-27	Saccharated Fe oxide given I. V.									
7-30	5.8	3.1	12.3		11,400	42		4	53	1
8-3	6.4		16.4							
8-12	7.8		6.4		19,400	26	2	3	67	2
8-18	8.1		2.8							
9-9	11.6	4.8	3.6	454,000	25,400	14		2	85	
9-17	10.4				14,800	12		2	86	
10-11	10.4				11,250	33		3	64	
11-12	10.0	3.97	0.9	550,000	10,050	22	1	1	76	
12-6	10.4	5.02			7,200	61			39	
Case 2										
11-24	5.0	3.1			13,500	32	1	3	61	3
11-26	5.0	3.0	2.9	270,000	8,300	42	2		56	2
12-1	4.6		3.1		13,800	28	2	4	61	5
12-2	4.0		2.89		15,700	26	4	3	62	5
12-6	5.6		2.5		16,000	30	2	3	57	8
12-15	Oral Fe medication started									
12-17	4.4		1.0							
12-18	6.0		11.0		16,000	15		8	71	6
12-20	10.0		5.0							
1-2-55	10.4		1.8		23,000	62	2		36	

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TABLE 8.—*Peripheral Blood Counts*—Continued

Case 3

Date (1955)	HGB Gm./ 100 Ml.	RBC M³	Retic %	Plat M³	WBC M³	WBC Differential				
						PMN	Band	Eos.	Lymph.	Mono.
6-30	5.8	2.29	2.5		12,970					
7-7	5.5	2.26	0.7	295,000	11,100	24	4	8	59	2
7-14	4.8	1.97	1.0		14,300	16		1	80	3
7-19	4.0	2.23	1.3	259,000	19,450	19		2	78	1
7-21	4.4	1.92	2.3		16,000	22		3	72	2
7-29	Oral iron medication started									
8-2	4.4	2.28	12.3		13,770	12	3	4	73	8
8-8	6.7	3.01	13.1		10,800	38			59	3
8-17	9.1	2.89	3.9							
8-22	11.3	4.12	3.1		11,920	23.2	3.6		70	3.2
9-14	8.4				10,750	44	4		46	6
10-25	11.2	4.5	0.6							
11-22	13.3									

Case 4

10-17	10.4	3.27	0.4	295,000	8,580	19	1		64	13
10-26	10.0	3.6	0.5		17,000	25	3		47	22
11-2	7.6	2.5	1.3		13,870					
11-7	7.2	2.45	3.3							
11-14	7.0	2.98	2.9		9,950					
11-17	7.1	2.52	1.6							
11-28	6.2		0.9							
12-19	6.6	2.81	1.4							
12-19-55	Oral iron medication started									
12-27	7.8	2.91	7.4							
1-3-56	10.5	4.42	1.4							
1-22	12.7									
3-13	12.7	4.18	0.4		11,200	25			66	85
3-28	11.0	3.87	0.2							
4-2	11.0	4.12	0.1		8,200	7.0	3	2.5	87.5	
4-16	10.8	3.72	1.1		10,750	20	1	2	72	

states was not present. An increase in the numbers of less mature erythroid precursors such as are encountered in hemolytic anemias was lacking. No evidence of megaloblastic erythropoiesis could be identified. The late normoblasts present in the marrow were relatively few in number and were unusually well hemoglobinized compared with the erythrocytes in the circulation (Fig. 8).

The numerical relationships of the cellular elements in the bone marrow are listed in Table 9.

The fasting serum iron level was determined in each patient shortly after being first seen with symptoms of disease. In each instance the serum iron level was distinctly low. The total iron-binding capacity of the serum was measured and was likewise found to be reduced; the range of values

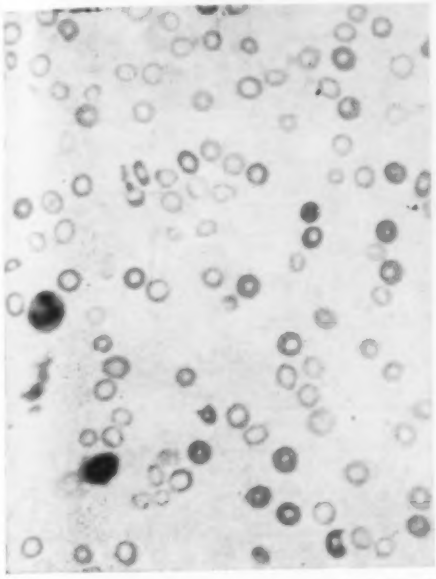

Fig. 6.—Photomicrograph of peripheral blood smear in Case 3. The microcytosis and hypochromia are evident; ×720.

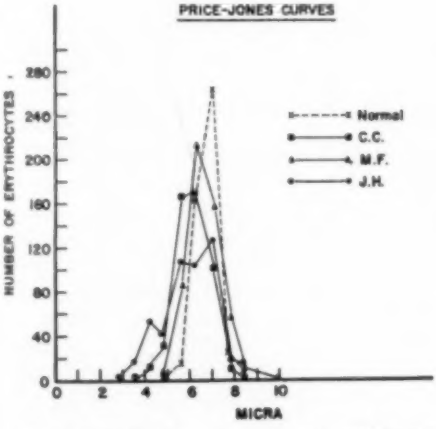

Fig. 7.—Price-Jones curves of erythrocyte diameter show the moderate degree of microcytosis found in each of three patients.

being 221γ per 100 cc. to 262γ per 100 cc. These levels, although distinctly reduced, were obviously not the limiting factor responsible for the low level of circulating serum iron. Unfortunately, our data are much too limited to be interpreted

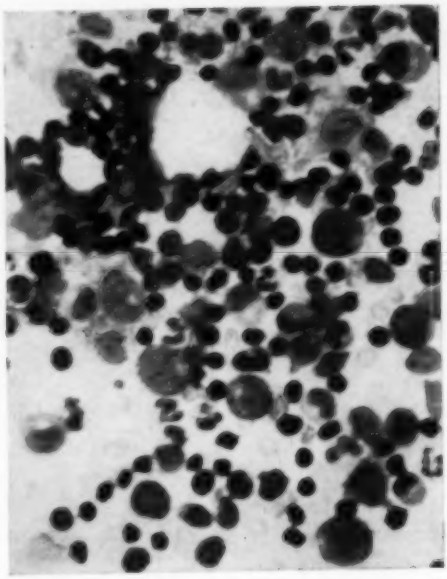

Fig. 8.—The bone-marrow-aspiration smear in Case 3. A marked lymphocytosis is present. The absence of megaloblastosis and normoblastic hyperplasia is referred to in the text; ×720.

as showing any trend in serum iron level with the levels of circulating hemoglobin or plasma protein.

In three of the four patients the serum copper level was reduced. The fourth patient (Case 1) was studied only in the recovery phase of her disease and on three occasions had normal or elevated levels of serum copper. The numbers of observations of the serum copper level are, unfortunately, limited in each individual patient but the level of the copper in the serum appeared to rise after the administration of albumin intravenously in one patient. In one other patient, Case 3, the plasma copper level rose to normal levels only after several weeks of observation and after administration of an oral medicinal iron preparation.

The free-erythrocyte-protoporphyrin levels were determined and found to be elevated in three of the four patients. There was poor correlation with the degree of elevation of the protoporphyrin level and the degree of anemia. The levels were elevated in each anemic patient throughout the period of ob-

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TABLE 9.—*Differential Counts of Bone-Marrow Aspirates*

Marrow Cells		Case 1	Case 3		Case 4
			7-7-55	10-3-55	10-27-55
Blast		0.8%	1.4%	2.0%	3.6%
Promyelocyte		3.8	2.2	6.8	5.2
Myelocyte	Neut.	10.0	8.4	20.2	11.6
	Eos.	2.6	3.2	2.6	5.6
Metamyelocyte	Neut.	11.2	12.8	22.4	16.2
	Eos.	1.2	3.6	2.0	2.8
Band	Neut.	14.6	13.4	15.6	8.6
	Eos.	1.0	.4	1.2	3.2
Polymorph.	Neut.	15.0	11.8	10.0	2.0
	Eos.	0.8	.4	2.0	2.2
Prerythroblast		3.6	2.8	1.4	1.2
Erythroblast		15.0	6.0	3.2	6.2
Normoblast		15.4	9.0	8.6	6.6
Lymph.		6.2	20.0	2.2	30.4
*Plasma cells per 5000 cells		36	34	21	27

* Plasma cells are counted per 5,000 nucleated cells. Four patients without abnormal serum proteins and less than one year of age had 20 plasma cells/5000 marrow cells.

servation. The lowest levels of free erythrocyte protoporphyrin were found in the patient in Case 1, who was studied only during the period of spontaneous recovery. The results of these chemical analyses are recorded in Table 10.

In view of the morphological findings in the circulating erythrocytes and the low serum iron in the presence of optimal intake of iron-containing foods, it was felt pertinent to determine whether or not iron absorption from the gastrointestinal tract was abnormal. One of the infants (Case 4) was thus fed egg containing Fe^{59} obtained from a hen previously injected intravenously with the isotope. The egg was scrambled and quantitatively fed as part of a normal breakfast (one half piece of toast, orange juice, and two ounces of milk). Quantitative collections of stools were carried out for five days until less than 1% of the radioactivity appeared in the stool. Eighty per cent of the ingested Fe^{59} was recovered in the stool. The 20% absorbed compares well with our normal controls of this age group.[29]

During the course of observation each of the four patients was treated with medicinal iron. In Case 1 200 mg. of saccharated iron oxide was given intravenously to test the hypothesis that this infant was not iron deficient. A prompt reticulocytosis and progressive rise in hemoglobin ensued. In the remaining three patients no iron medication was given until after several weeks of observation, and the hemoglobin in each instance had progressively fallen to less than 4.5 gm. per 100 cc. This severe anemia was due in part to repeated phlebotomies. Iron was given orally as a concentrated solution of ferrous sulfate (Fer-In-Sol) in a dose

TABLE 10.—*Serum Iron, Serum Copper, and Free Erythrocyte Protoporphyrin Determinations*

Date	Total protein, Gm./100Ml.	Hemoglobin Gm./100Ml.	Iron, Mg./100Ml.	Copper, Mg./100Ml.	Free Eryth. Proto-porphyrin	Total Iron Binding Capac., Gm./100Ml.
1954	Case 1					
7-14	3.5	7.1	32.1	206	33.1	
7-26	4.6	4.7	71	197	56.3	221
11-25		10.2	49	96.4	82.4	
1954	Case 2					
11-24	2.6	5.0	36.4	23.0	203	
12-1	3.6	4.6	41	72	319	241
1955	Case 3					
7-19	2.7	4.0	28.4	31.3	288	228
7-22		4	22.3	11.76	124.7	
10-24	4.7	7.1	12.1	125.4	18.2	
1955-56	Case 4					
10-26	3.2	10.0	22.3	43.2	135	
11-2	5.3	7.6	12.1	121.52	93.4	262
12-19	4.8		14			
1-17	5.0	12.1	63	129		
3-13	5.3	12.7	151			

which provided 48.0 mg. of elemental iron in four divided doses each day. Each infant responded to the medication with prompt reticulocytosis and progressive rise of hemoglobin concentration to normal. The serum iron and copper levels were not determined at frequent intervals and thus the effect of iron therapy on these values cannot be adequately evaluated. In the third infant a normal copper level was observed for the first time after 11 weeks of oral iron therapy. The effect of the iron in increasing hemoglobin production was not altered by the protein level or the ratios of albumin and globulin in the serum. In two infants hypoproteinemia of marked degree persisted during the period of the rising hemoglobin level. In a third infant moderate reduction of the serum proteins (4.8 to 5.1 gm. per 100 cc.) persisted during this period. In the infant given the iron intravenously, the protein level was increasing when the material was injected and continued to rise to normal levels. In none of the four patients could the iron medication be identified as favorably increasing the levels of serum protein. The persistent lymphocytosis noted in all of the patients was not altered by the administration of therapeutic iron.

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General Comment

The age at which the hypoproteinemia appeared in these infants is a period when relatively low levels of plasma proteins are common in normal infants. Evidence from several sources is available to suggest that the maternally contributed fraction of the infant's serum protein is slowly metabolized and is undetectable after age 2 to 3 months. In addition, Good has found that an infant who received no gamma globulin from its mother (who suffered from agammaglobulinemia) showed no detectable gamma globulin in its blood until the age of 3 months.[30] These findings suggest that the rate of synthesis of plasma protein in infants under 3 months is either extremely slow or delayed in onset. The adult level of gamma globulin is not demonstrable in many infants until age 2 years, which suggests that a low level of the plasma protein fraction alone is not enough to initiate a rapid production rate. The finding of retarded "production rates" of albumin, and beta globulin in the very young infant, may be the result of "hepatic immaturity" which has been shown to exist as regards other liver functions. During infancy, a maximal rate of protein production by the hepatic cell may be inadequate to maintain adult concentration of plasma proteins in addition to meeting the needs for synthesis of proteins for other uses, such as organ growth. Since the gamma globulin fraction has its origin in the plasma cell and not the hepatic cell, a more generalized process than hepatic immaturity is suggested. Since the microsomal fraction of certain cells appears to be the origin of plasma protein, some enzyme system common to microsomes of both hepatic and plasma cells would appear to be involved when all serum protein fractions are inadequately produced. The role of serum protein degradation in normal infants is not completely settled by evidence at hand, however. Maternal protein appears to be degraded at a normal adult rate by the infant, and after its disappearance only a slow rise of the infant's proteins to normal concentration levels ensues. The dynamic equilibrium of normal metabolism of infant protein may escape detection, however, if concentrations rather than rates of production and degradation are measured. The production of a modified protein molecule during early infancy could conceivably result in a shortened survival time of endogenously produced serum proteins. A low level of serum protein concentration could result from a dynamic imbalance of this type as well as from a decreased rate of synthesis alone.

It seems conceivable that the greater than normal hypoproteinemia in the four cases reported here could be an exaggeration of a normal mechanism. These infants did not manifest edema in the early newborn period, which suggests that the pathologic level of serum protein developed after disappearance of the maternally acquired proteins. In the presence of an increased rate of serum protein degradation, the rate of protein synthesis has become inadequate to prevent hypoproteinemia, even though this production rate is equal to that found in normal infants. The reason for this increased plasma protein turnover rate is not apparent from our studies. As the infant grows older and more mature, the microsomes may be enabled to increase the plasma protein production rate to one which will result in normal concentrations of circulating protein. Proportional decreases in albumin and all globulin fractions is not usually found in hypoproteinemia resulting from other commoner causes. The hypoproteinemia due to dietary protein deficiency, the nephrotic syndrome, cellular damage to the liver, and inflammatory processes is usually characterized by a reversal of the albumin/globulin ratio, which has not been evident here.

The cellular origin of gamma globulin is different from that of albumin. Thus, it does not seem likely that the mechanism responsible for the hypoproteinemia in these patients is similar to the isolated defect in the plasma-cell production of protein found in patients with congenital agammaglobulinemia. The reduction in total protein in

congenital agammaglobulinemia is small, and can be accounted for entirely by the absence of the gamma fraction.[31] It is of interest that each of these patients was found to have plasma cells present in normal numbers in the marrow aspirate. It is, of course, possible that a single enzyme defect could be present in several types of cells and thus account for the decreased serum concentrations of all albumin and globulin fractions. Normal somatic growth rates and over-all nitrogen balances indicate compensated or normal protein metabolism at the sites of production of these other proteins. If, however, one assumed a near-normal rate of plasma protein production accompanying a rapid destruction of albumin and globulin fractions by the same process, the same lowering of plasma protein concentration could result. This latter hypothesis seems more tenable, and that such a derangement is present in the patients considered here is better supported by the evidence presented.

Although transitory edema of the newborn infant is occasionally observed, it is quite unusual that an infant past the neonatal period should develop obvious clinical edema. When such causes as proteinuria, malnutrition associated with intestinal malabsorption or severe underfeeding, and hepatic diseases such as cirrhosis of infancy are eliminated, hypoproteinemia during the first year of life is very rare.

Severe anemia in early infancy is not a common occurence and when encountered a variety of etiological factors must be considered as possible causes of the hemoglobin deficit.

In the patients considered here the absence of icterus, clinical evidence of increased pigment excretion, significant splenomegally, hyperbilirubinemia, and reticulocytosis convincingly support the premise that the anemia was not due to increased destruction of erythrocytes. Loss of erythrocytes by hemorrhage is not supported or suggested by any of the clinical or laboratory data.

Inadequate production of erythrocytes would appear to be a more plausible explanation for this deficit of circulating hemoglobin, i. e., anemia. The presence of striking hypochromia in all of the patients would suggest that the production deficit is more particularly affecting hemoglobin production than erythrocyte production per se.

The morphological findings in aspiration material from the bone marrow in each infant demonstrates an adequate number of normal-appearing hematopoietic elements to be present in the marrow of each patient. Although compensatory increases in erythroblastic elements could not be demonstrated in these patients, the marrow and the hematopoietic tissues appeared to be intact and structurally capable of normal blood-cell production.

In light of these morphological findings, the possibility that the erythropoietic tissues were not being provided with adequate supplies of the precursors for normal hemoglobin and erythrocyte precursors is immediately suggested. The supply of such nutrients could be inadequate in the presence of environmental factors making ingestion of a normal dietary intake impossible or might be inadequate if certain intrinsic abnormalities existed which prohibited the normal metabolism of necessary nutrients even though they were ingested in normal amounts.

The diets of the patients had been optimal by all current standards, had been taken well, and appeared to be assimilated in a normal manner as shown by the balance studies reported in the previous section. Our observations concluded that in no way did the diets of these children differ from diets of thousands of other normal children in this community who are maintained in apparently adequate nutrition on such feedings.

Iron depletion is the most commonly encountered nutritional deficit responsible for impaired hemoglobin synthesis. The hypochromic, microcytic erythrocytes, the low serum iron level, and the prompt response of each infant to iron medication are in keeping with the conclusion that anemia

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encountered here is due to iron deficiency. The age of onset of the anemia in the absence of demonstrable blood loss, the low serum copper, the good dietary intake, and the absence of normoblastic hyperplasia in the bone marrow are not in keeping with the conclusion that the hemoglobin deficit resulted from lack of available iron from extrinsic sources. That dietary iron was normally available and could not be assimilated from the intestinal tract does not seem to be a plausible explanation for the possible occurrence of iron depletion. Infants of this age probably do not have a large demand for exogenous iron, and severe anemia due to lack of dietary iron should not occur in very early infancy in the absence of blood loss. In one patient the quantitative determination of the absorption of iron from egg clearly demonstrated that normal amounts of iron were assimilated from foods in the diet.

The finding of hypocupremia in the three of the four patients who were seen during prolonged symptomatic periods of their disease is of considerable interest. Low levels of copper in the serum have been found only in the newborn, hepatolenticular degeneration, nephrotic syndrome, sprue, and in severe hypoproteinemia.[32] The abnormalities responsible for the low level of circulating copper in these disorders and in the newborn are not clear. In the nephrotic syndrome large amounts of protein-bound copper are lost in the urine, in sprue defective absorption of copper might be responsible, and in Wilson's hepatolenticular degeneration and the newborn no plausible explanation for the low copper values is apparent. It is important to appreciate that low serum-copper values are not in themselves any indication of the status of body-copper content and stores. In both the newborn and in hepatolenticular degeneration the serum copper concentrations are low, and yet it is known that in both these instances the tissue content of copper is very high.

The low serum-copper values found in the patients discussed here might be explained as merely a reflection of the low globulin content of the plasma. Copper exists only bound to protein in the plasma, chiefly alpha ^{2} globulin. It seems reasonable that the very low level of this plasma protein fraction would appear to be responsible for the low copper levels found. The sparse data available appear to show a direct correlation between the levels of plasma protein and copper levels.

That the hypocupremia could be a manifestation of copper depletion resulting from exhaustion of neonatal copper stores and inadequate dietary intakes of the metal, seems highly unlikely. The data concerned with dietary copper requirements of growing infants are scanty and variable. Figures from growing school children obtained in balance studies by Scoular[33] and by Macy[34] suggest that about 80γ/kg. of body weight might represent the normal daily requirement. Information regarding the copper content of commercially available infant foods is not available at present. Cows milk contains 0.1 to 0.5 mg/liter, with the lowest values being present during winter months. It is stated that most foodstuffs are rich in copper and that even mediocre infant diets contain more than adequate amounts of this element. However, until the copper content of commercial baby foods is known, one cannot justify this assumption.

Bush[32] refers to an attempt by Lahey to produce copper depletion in two infants in a hospital environment by limiting the diet to especially prepared low-copper-containing milk. Over a period of four to five months he was unable to produce hypocupremia, anemia, or other signs of nutritional deficiency.

Cartwright[35] and his associates have produced copper deficiency by dietary means in rapidly growing swine. Anemia was a prominent feature of the deficiency state. The anemia could not be distinguished on a morphological basis from the hypochromic, microcytic, reticulocytopenic picture found in iron depletion. The marrow findings were that of normoblastic hyperplasia. The

serum-iron as well as the serum-copper values were low. There were no alterations in the plasma protein. There was a prompt response to copper supplementation with increases in the reticulocytes, the hemoglobin, serum iron and copper levels.

Several distinct differences in the hematological findings are apparent between these copper-deficient animals and our findings. The serum-iron levels did not rise in association with the increase in plasma-copper levels. The bone-marrow picture was distinctly different in the patients where increased normoblastic activity was not found. From this accumulated evidence it is difficult to assume that the hypocupremia found in the patients studied here was other than a secondary manifestation of hypoglobulenemia and not a manifestation of copper depletion in these infants.

Other nutrients, of intrinsic or extrinsic origin, essential for hemoglobin synthesis might well be considered in an attempt to elucidate factors responsible for the unusual anemia encountered in these infants. The dietary intake of proteins and vitamins was adequate for normal subjects, but that a relative inadequacy might have been present for some other essential nutrient must be considered. Such a situation might exist in the presence of excessive rates of excretion or the defective metabolism of a nutrient.

Cartwright has recently reviewed the literature concerning the hematopoietic consequences of deficiencies in the vitamins and other trace nutrients.[36] It would appear that of the many vitamins only niacin, pyridoxine, riboflavin, pantothenic acid, ascorbic acid, folic acid, and vitamin B_{12} play a demonstrable role in erythropoiesis. Dietary deficiencies of none of the B vitamins except folic acid have been encountered as clinically responsible for anemia in man. In animal experiments the hematological alterations associated with induced deficiencies of these substances are in no way comparable to the findings in the clinical material considered here. Likewise, the megaloblastosis associated with the vitamin C, folic-acid, and vitamin-B_{12} deficiencies were not encountered in the patients.

That protein and its constituent amino acids are necessary for normal erythropoiesis and hemoglobin production has been known for many years. Adequate studies of the hematological alterations in human material associated with pure protein deficiencies are not currently available. It is well established that hypoproteinemia and moderate degrees of oligemia will follow periods of protein starvation. However, anemia is often difficult to define in such situations, since reductions of the total circulation erythrocyte mass are not demonstrated in the reduced plasma volume present if only concentrations of hemoglobin and erythrocytes are determined.[37] Anemias in animals on low-protein intake are of interest in regard to the current clinical material, since the morphological characteristics in the peripheral blood and bone marrow are not unlike those encountered in our patient.

In protein deprivation hypochromia, microcytosis, and reticulocytopenia are common with a moderate degree of lymphocytosis present. The marrow has been best studied in animals by Brown[38] and is characterized by erythroid and normoblastic hypoplasia and eosinopenia. These morphological findings in the four infants of interest here are suggestive of protein deprivation of moderately severe degree.

In face of an obviously normal intake of protein, and without evidence of protein loss in the urine, one cannot attribute the hematological alterations to protein deprivation without further consideration. It is to be remembered that protein deficiencies may be qualitative as well as quantitative and may be due to intrinsic environmental abnormalities as well as extrinsic factors which might exclude normal protein from the diet.

It has been impossible for us to define an extrinsic deficit, quantitative or qualitative, in the protein intake in any of the four patients, and one would thus postulate that some metabolic abnormality exists preventing the normal nutrition of the hematopoietic tissue with protein and protein

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constituents. An obvious area to explore is the loss from the body of an amino-acid essential for hemoglobin production, isoleucine, lysine, or tryptophan.

There was a reticulocyte and hemoglobin response in each patient upon administration of medicinal iron. An adequate explanation for this finding is not apparent. It is of interest that Zuelzer has observed an unusual patient with persistent hypoproteinemia and persistent hypochromic anemia.[39] A prompt hemoglobin response followed the administration of iron to this patient on repeated occasions with recurrence of anemia when iron medication was discontinued. This patient obviously was not suffering from iron depletion. He was unable to utilize body-iron resources for hemoglobin synthesis, although iron in large amounts from the gastrointestinal tract was readily utilized for production of hemoglobin. It is tempting to speculate that a similar situation was present in the four patients presented here. Until the metabolic interrelationships of protein and iron are more clearly defined the significance of these interesting observations will be obscure.

In the past 10 years, two dramatic episodes have occurred to emphasize our incomplete knowledge of the nutritional requirements of infants. Megaloblastic anemia of infancy was the first of these. Studies which led to a better understanding of the role of folic, folinic, and ascorbic acids, as well as vitamin B_{12} in normal hematopoiesis were stimulated. The second episode confirmed that pyridoxine must be supplied to the growing infant. It should be pointed out that although much knowledge of all these nutrient factors was available it remained for the clinical studies of infants accidentally deprived of them to lead the way to their being finally established as required factors. Careful dietary histories on each infant did not reveal either a common type of milk preparation or a common source of solid foods. Each infant had received a diet determined as adequate in all known essential nutrients. Indeed, each infant was hospitalized during the illness and was thus personally observed to consume the adequate diet. Two of the patients (Case 1 and Case 2) began to improve almost immediately when removed from their homes. Since these were the first two infants studied, it was tempting to assign their improvement to either removal from a toxic influence or introduction of a missing essential occasioned by their entrance into the hospital. The remaining two infants, however, discouraged speculation of this nature. These latter infants failed to improve until they were again returned to their homes after periods of hospitalization. The age at which all infants improved seems more constant than does the place of beginning improvement. One infant (Case 4) was given parenteral doses of pyridoxine without effect. No other attempts at specific therapy were made, since we felt unable to accurately predict the time of spontaneous recovery which could easily be mistaken for a result of any type of therapy. In spite of a lack of clear-cut evidence in favor of an isolated deficiency of an essential nutrient, little evidence is available to deny such an etiology.

The possible role of copper deficiency in such patients has been suggested by Sturgeon and Leahy. Although the close association of this metallic ion with protein in the blood is well recognized, no evidence is available to suggest its role in protein formation or degradation. Cannon has shown that when animals are chronically deprived of potassium that serum protein levels become low. Although potassium balances were positive in two of these patients, little is known of the status of trace elements in these patients. It is assumed that no deficiency existed, since the diets they received were essentially those received by thousands of healthy American infants.

This same reasoning must be applied to the status of essential amino acids in these infants. Although it is conceivable that some unusual and unrecognized circumstance in some way decreased the content of a single essential amino acid in the diet of these infants, it does not seem at all probable.

Chronic urinary loss of an essential amino acid is a possibility. However, the studies of protein dynamics suggest a rate of degradation which is rapid rather than a failure of synthesis. Deficiencies of essential amino acids appear to retard synthesis and thus reduce protein concentrations. Aminoaciduria is also found in such deficiencies, since other amino acids are not utilized when one essential amino acid is missing.

Summary and Conclusions

In an 18-month period, four infants under 8 months of age with an unusual syndrome of edema, irritability, and pallor were studied. Each infant was born at term, had ingested an optimal diet, and had no laboratory evidence of renal or other hepatic disease. Marked hypoproteinemia was present in each patient at some time during the illness. The age of onset of symptoms ranged from less than 1 month to 7 months. The disease was apparently self-limited with spontaneous recovery occurring in each infant between the 7th and 9th months of life. The striking similarity of the clinical findings of these patients suggests a single disease process which might be ascribed to a fundamental defect in protein metabolism.

The hypoproteinemia was characterized by a uniform reduction of the concentration of albumin and all globulin components. Continued normal growth rates in all patients as well as the nitrogen balance studies performed in two patients indicated that the over-all nitrogen balance was positive. Balance studies also revealed that the urinary excretion of nitrogen was high in comparison to the dietary intake of protein. The excretion of an apparently normal amount of isotopic nitrogen in the urine after the ingestion of glycine labelled with N^{15} in one patient suggested that the increased urinary excretion of total nitrogen was not the result of a failure of utilization of dietary nitrogen.

Studies of plasma protein turnover rates were made in three of the infants using tracer doses of phenylalanine-3-C^{14}. Two normal infants were also studied in a similar fashion. The rate of incorporation of this isotope in each protein fraction was similar in the hypoproteinemic and the normal infants. Of particular importance was the finding that the isotope label disappeared from the circulating protein at an increased rate in the infants with hypoproteinemia. This finding was most striking in the albumin fraction. It would appear that this increased rate of plasma protein degradation and the failure to compensate for it with an increased rate of production is responsible for the low circulating levels of albumin and globulin in these infants.

Further evidence of this rapid rate of protein turnover was gained from the immunologic studies. Gamma globulin could not be demonstrated in the plasma of these infants by Tiselius or paper-strip electrophoresis. One of the infants was able to respond to antigenic stimulation with the production of antibodies measurable by immunologic techniques. During a period when no gamma globulin was evident by other techniques, all patients showed a normal number of plasma cells in the bone-marrow aspirate.

The plasma albumin of two patients was compared in its antigenic properties with albumin from normal infants and adults, by the Ouchterlony agar plate precipitin technique. The major antigenic component of the albumin obtained from the infants with hypoproteinemia was identical with that from normal infants and adults.

Anemia was present or developed in each of the four patients. Microcytosis and hypochromasia of the erythrocytes and a moderate lymphocytosis characterized the cellular morphology of the peripheral blood. Hypoferremia, hypocupremia, and a reduction in the iron binding capacity of the plasma were found. There was elevation of the free erythrocyte protoporphyrins. Bone-marrow aspiration biopsy failed to reveal the normoblastic hyperplasia characteristic of simple iron-deficiency anemia. Bone marrow morphology was otherwise normal.

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The intestinal absorption of iron was within normal limits, as demonstrated by an iron balance study using Fe^{59}-labelled eggs as a dietary source of iron.

Although the dietary history, age of onset, bone-marrow-aspiration findings, and blood chemistries did not indicate that the anemia was due to iron depletion, the hemoglobin levels rose in each patient following the administration of medicinal iron.

The extensive studies reported here on a group of four infants suffering from hypoproteinemia and anemia of a transitory nature suggest that an abnormality in protein metabolism at the cellular level is responsible. The normal dietary intakes during the development of anemia and hypoproteinemia and the spontaneous return to normal without recognizable changes in the diet as well as the many studies presented here indicate that this disturbance was not primarily of dietary origin. Other possible etiologies have been discussed. The evidence at hand does not permit assignment of a definite etiologic factor, although the mechanism for the development of signs and symptoms seems clear.

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Copper Deficiency in Infants

A Syndrome Characterized by Hypocupremia, Iron Deficiency Anemia, and Hypoproteinemia

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Introduction

Low serum copper concentrations are characteristic of the newborn infant * and are known to occur in nephrosis,† in sprue,[7] and in the hepatolenticular degenerative disorder of Wilson.‡ In addition, Zurukzoglu-Sklavounou in Switzerland,[11] Stransky in the Phillipine Islands[12] and Axtrup in Sweden,[13] although attaching little or no significance to the findings, have published data indicating the sporadic occurrence of hypocupremia in infants with hypochromic, microcytic anemia. On the other hand, it is generally concluded that the serum copper concentration is normal or elevated in iron deficiency anemia and data supporting this position have been reported by Axtrup,[13] Brenner,[14] Freudenberg,[15] and by Lahey and co-workers,[9] as well as by the previously cited authors.§

Infants maintained on nearly exclusive milk diets are likely to develop a microcytic, hypochromic anemia typical of iron deficiency and closely resembling the anemia produced in swine by copper-deficient diets.‖ Hypocupremia, indicative of a nutritional copper deficiency, might be expected to occur in these infants, since both bovine and human milk have low copper concentrations,¶ and since the copper requirement of the rapidly growing infant might be expected to be high. However, Bush cites experiments which failed to produce hypocupremia by feeding two infants, one 5 days and the other 8 months of age, for 4 and 5 months, respectively, diets identical to those used to produce copper deficiency in swine.[20]

In our clinic, five infants with hypochromic anemia and laboratory findings of marked hypocupremia, hypoferremia and hypoproteinemia have been studied. The preliminary data suggest that these cases represent a multiple deficiency syndrome previously unrecognized.

Methods

The routine hematologic methods employed have been described elsewhere.[4] The method used for the serum copper concentration has been described by Gubler and associates[21]; that for the serum iron by Hamilton and co-workers[22]; for the serum iron-binding capacity by Rath and Finch,[23] and for free erythrocyte protoporphyrin by Grinstein and Watson.[24] Normal values for adults and children with the use of the above procedures and for the routine hematologic determinations employed in this laboratory have been reported.[4]

Filter paper electrophoretic studies of the serum proteins were performed using the Spinco Model

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* References 1-4.
† References 5 and 6.
‡ References 8-10.
§ References 11 and 12.
‖ References 16 and 17.
¶ References 18 and 19.

R apparatus. Technical details of the procedure are as follows: buffer, barbital, pH 8.6, ionic strength 0.075; 0.01 ml. of serum applied to each strip; migration, 16 hours at constant current of 5 ma. per eight-strip cell. After migration the proteins were heat-coagulated, stained with bromophenol blue, and fixed with sodium acetate. A Spinco Analytrol, a servo-type integrating scanner, was used to measure the relative concentrations of the protein fractions as indicated by the amounts of dye present on the filter paper strips.# Total protein and albumin and globulin concentrations in the serum were determined by a slight modification of the salt fractionation and biuret method of Kingsley.[20]

Report of Cases

CASE 1.—A 9-month-old white girl was admitted to the Los Angeles Childrens Hospital on Dec. 22, 1952, with a history of periorbital edema for 3 weeks and generalized edema of 4 day's duration. She was the product of a full-term first pregnancy. Her birth weight was 3200 gm. (7 lb.). The mother had "hemorrhaged" during the first trimester, but the remainder of the pregnancy was regarded as normal. Delivery was spontaneous after a five-hour, uncomplicated labor. The infant's development was normal and the only past illness had been one minor upper respiratory infection at the age of 3 months. There were no symptoms of urinary-tract disease or disorders of the gastrointestinal system. The infant had been breast-fed for one and a half months, then changed to an evaporated milk formula. At the time of admission, her diet was principally whole milk (18-24 oz/day), although strained foods and cereal reportedly were taken fairly well.

Physical examination revealed generalized edema, most pronounced over the dorsum of the hands and feet and in the periorbital region. Marked pallor was noted. The remainder of the physical findings were within normal limits. Laboratory findings on entry are tabulated in the first line of Table 1. These revealed a marked hypoferremia (serum iron, 18γ/100 ml.), hypocupremia (serum copper, 43γ/100 ml.), and hypoproteinemia (albumin, 1.7; globulin, 1.0 gm/100 ml.). Additional hematologic studies revealed a microcytic (mean corpuscular volume, 74μ³), hypochromic (mean corpuscular hemoglobin concentration, 28%) anemia. Urinalysis was negative. The blood nonprotein nitrogen was 32 mg/100 ml.; the serum cholesterol, 149 mg/100 ml., and the sulfobromophthalein retention was 4% at 40 minutes.

Treatment consisted of one transfusion of 50 ml. of sedimented red cells, the oral administration of ferrous sulfate, approximately 500 mg. per day, and the inclusion of solid foods in the diet. The changes in laboratory findings following the above therapy are listed in Table 1. Within two weeks, on 1-5-53, the edema subsided, the hemoglobin concentration increased 1.4 gm. per 100 ml., and the total serum protein and the albumin and globulin concentrations returned to within the normal range. A follow-up examination approximately two and one-half years after discharge from the hospital, on 6-17-55, showed all of the above values within the normal range.

CASE 2.—A 7½-month-old Negro girl, who had been noted to have swollen eyelids for one week and a swollen left arm for one day, was admitted to another hospital on 3-27-55 and seen by one of us (P.S.) in consultation three days

TABLE 1.—*Summary of Hematologic Data,* Case 1*

Date	Hb., Gm/100 Ml.	RBC, 10⁶/Mm.³	S. I., γ/100 Ml.	T. I. B. C., γ/100 Ml.	% Sat.	S. C., γ/100 Ml.	E. P., γ/100 Ml.	Serum Proteins, Gm/100 Ml.		Remarks
								Alb.	Glob.	
12-23-52	7.0	3.38	18	368	5	43		1.7	1.0	
12-29-52								3.3	1.6	Transfusion of 50 ml. sedimented red blood cells
1- 5-53	8.4	3.23						4.4	1.5	
6-17-55	12.0	4.6	131	431	30	148	19	4.8	2.1	

*S.I.=Serum Iron; T.I.B.C.=Serum Total Iron Binding Capacity; % Sat.=Per cent Saturation of Total Iron-Binding Capacity by the Serum Iron; S.C.=Serum Copper; E.P.=Free Erythrocyte Protoporphyrin.

See Operating Instructions, Spinco Model R, Paper Electrophoresis System, Spinco Division of Beckman Instruments, Inc., Belmont, Calif.

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later.† For two days prior to admission there had been occasional vomitting and the infant was listless and irritable. She was the product of a full-term, third pregnancy. The mother was reported to be anemic during the pregnancy, but this was believed to have been corrected before term. The patient's development had been normal. One mild upper respiratory infection and an infected toenail at 4 months of age were the only past illnesses. There were no symptoms of urinary-tract or gastrointestinal disease. The infant's diet consisted principally of homogenized milk; solid foods were refused.

Physical examination upon admission to the hospital revealed moderate pallor of the skin and mucous membranes, periorbital edema, and edema of the left forearm and hand. The remainder of the physical findings were regarded as normal.

Laboratory findings on entry are tabulated in the first line of Table 2. Marked hypoferremia (serum iron, 5γ/100 ml.), hypocupremia (serum copper, 28γ/100 ml.), and hypoproteinemia (alb., 2.9; glob., 1.2 gm/100 ml.) were present. Additional hematologic studies revealed a microcytic (mean corpuscular volume, 61μ³) anemia. Urinalysis was negative. The 24-hour total urinary protein excretion was 4 mg. The blood urea nitrogen was 14 mg/100 ml.; the thymol turbidity 2.6 units, and cephalin flocculation was negative at 24 and 48 hours.

The infant's condition remained unchanged for the first three hospital days. On the fourth day the diet was changed to a meat-base formula.‡ On the following day, temperature elevation, repeated vomitting, and heart failure evidenced by tachycardia and sudden occurrence of hepatosplenomegaly were noted. The infant was digitalized and given a transfusion of 90 ml. of sedimented red blood cells. The signs of cardiac decompensation and other symptoms improved within 24 hours, and the patient was discharged on the 10th hospital day. The subsequent course has been uneventful. Laboratory tests performed during hospitalization and on follow-up visits are tabulated in Table 2. One month after entry (April 25, 1955) the hemoglobin concentration had risen to 10.6 gm. per 100 ml.; the serum iron and serum copper concentrations were in the normal range, and the free erythrocyte protoporphyrin concentration had decreased considerably. The serum albumin and globulin concentrations were found to be within the normal range two months (May 25, 1955) after the patient was first seen. Subsequently, her course has been uneventful.

TABLE 2.—*Summary of Hematologic Data,* Case 2*

Date	Hb., Gm./100 Ml.	RBC, 10⁶/Mm.³	S. I., γ/100 Ml.	T. I. B. C., γ/100 Ml.	% Sat.	S. C., γ/100 Ml.	E. P., γ/100 Ml.	Serum Proteins, Gm/100 Ml.		Remarks
								Alb.	Glob.	
3-27-55	6.2	3.43						2.2	1.6	Determinations at referring hospital
3-30-55	7.8	3.78	5	360	1.4	28	338	2.9	1.2	Periorbital edema
4- 4-55	8.2		23	478	5	48		2.8	1.1	Meat-Base Formula; transfusion 90 ml. sedimented cells
4-25-55	10.6	5.51	117			111	182			
5-25-55	11.9	5.9	36			142	120	4.3	1.5	
6-20-56	12.0	5.6	63			159	104	4.1	1.5	
2-13-56	10.5	5.6	52	413	12	196	132	4.2	2.3	

*For explanation of headings, see footnote to Table 1.

CASE 3.—A 6½-month-old Caucasian girl was admitted to the Los Angeles Childrens Hospital on May 2, 1955, with a history of edema of the face and eyes for the preceding week. She was the product of a full-term, third pregnancy. The pregnancy reportedly had been complicated by

† Drs. H. Bonney and I. D. Goldenberg, of the Southern California Permanente Medical Group, brought this case to our attention and have cooperated in following her progress.

‡ Meat-Base Formula manufactured by Gerber Products Co., Fremont, Mich. This product contains per 100 gm. approximately 7 gm. of protein derived from beef heart; other ingredients include sesame oil, sucrose, tapioca, calcium citrate, tricalcium phosphate, and vitamin D_3. As ordinarily prescribed this product is diluted with an equal quantity of water.

elevation of blood pressure. The infant was delivered with forceps after a 15-hour labor. Birth weight was 4056 gm. (8 lb. 15 oz). Her development had been normal. There had been no previous illnesses, and there was no history of symptoms referrable to urinary or gastrointestinal-tract disease. The infant had been breast fed until about 4½ months of age, then changed to whole milk feedings. At the time of admission she consumed 32 to 48 oz. of milk per day; solid food intake was poor.

Physical examination showed a pale, obese, irritable infant with edema of the upper eyelids. Minimal pretibial edema was noted also. A systolic murmur was heard along the left sternal border. The liver was palpable 1 cm. below the right costal margin. The remainder of the physical examination was normal. Laboratory findings on admission are tabulated on the first line of Table 3. These showed marked reduction in the concentrations of serum iron (4γ/100 ml.), serum copper (37γ/100 ml.) and serum proteins (alb. 2.1; glob., 0.8 gm/100 ml.). There was also reduction in the mean corpuscular volume (71μ³) and mean corpuscular hemoglobin concentration (24%). Urinalysis was negative. The blood nonprotein nitrogen was 32 mg/100 ml.; the serum cholesterol, 132 mg/100 ml.; the thymol turbidity 5 units, and the cephalin flocculation was negative at 24 hours and ± at 48 hours.

A one-week period of observation beginning May 2 was followed. During this time the infant was fed exclusively homogenized milk obtained from the same source as that used by the mother. In the middle of this period a mild diarrhea developed for which penicillin and streptomycin were administered. At the end of that time the orbital and tibial edema had cleared but the child looked extremely pale and showed clinical evidences of hemoconcentration. Blood studies at that time (5-10-55) are listed in Table 3.

To correct the anemia and hemoconcentration but to minimize alterations in the abnormalities of copper and globulins by parenteral therapy, a special transfusion was prepared. One hundred twenty milliliters of packed erythrocytes washed two times with two volumes of isotonic saline were reconstituted with 120 ml. of saline containing 15 gm. of human serum albumin. Analysis of this "blood" revealed a packed cell volume of 50%, "serum" iron concentration of 770γ/100 ml. and "serum" copper concentration of 162γ/100 ml. Of this material, 180 ml. was administered to the patient. Studies of her blood two days later

TABLE 3.—*Summary of Hematologic Data,* Case 3*

Date	Hb., Gm/100 Ml.	RBC, 10^6/Mm.^{3}	S. I., γ/100 Ml.	T. I. B. C., γ/100 Ml.	% Sat.	S. C., γ/100 Ml.	E. P., γ/100 Ml.	Serum Proteins, Gm/100 Ml.		Remarks
								Alb.	Glob.	
5- 3-55	5.2	3.11	4	304	1.3	37	324	2.1	0.8	Periorbital and pretibial edema; milk diet continued
5-10-55	5.9	3.67	14	308	2.6	63		3.6	1.2	180 ml. "special blood" transfusion
5-12-55	8.9	4.3	40	295	13.6	43		3.7	1.1	$CuSO_4$ 0.5 ml. 1% solution orally started 5-18-55
5-19-55			29			82		4.2	1.3	
5-23-55			16			111		3.7	1.4	
5-25-55	8.7		16			130		3.9	1.3	
5-31-55	8.3	4.75	16	496	3.2	225		3.6	1.9	Meat-Base Formula & $FeSO_4$ 0.3 gm/day started
6- 6-55	8.1	4.22	103	503	20.5	188		3.6	1.9	
9-23-55	5.2	3.89	9	589	2.0	142	225	3.8	1.2	
2-18-56	11.6	4.71	54	414	13.0	122	63	4.8	1.9	

*For explanation of headings, see footnote to Table 1.

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(5-12-55) revealed a 3-gm. increase in the hemoglobin concentration, a suggestive increase in the serum iron, and no change in the copper or protein concentrations (Table 3).

A moderately severe, nonspecific diarrhea was present from May 10, 1955 to June 30, 1955. During this time, when the diarrhea became severer, it was necessary to administer parenteral fluids and to change the feedings to boiled skimmed milk. The diarrhea continued intermittently for approximately eight weeks, until June 30. During a period when it had subsided (May 18 to 26), copper sulfate (0.5 ml. of a 1% solution) was administered orally each day. The rapid increase in her serum copper concentration associated with this therapy is shown in Table 3.

During the fourth to sixth weeks of this period (June 1 to 14, 1955) the diarrhea subsided. Meat-Base Formula, 24 to 30 oz. per day, and ferrous sulfate, 0.5 gm. per day, was given by mouth. However, after June 14 the diarrhea increased in severity and the patient was again treated with parenteral fluids and a transfusion of 100 ml. of whole blood. Feeding was resumed with protein milk, then boiled skimmed milk, and subsequently a banana-base formula was given. After this treatment the child was clinically well.

During and immediately after this period, observations pertinent to this investigation were not practical and the child was not studied until Sept. 23, 1955, when she was again admitted to the hospital with severe iron deficiency anemia. During the interval from June 30 to Sept. 23, the child was regarded as well. Her diet included adequate amounts of protein and mineral-containing foods. The milk intake was limited to one quart per day. There had been no recurrence of diarrhea. No evidence of blood loss had been noted.

On her second admission to this hospital, on Sept. 23, 1955, marked pallor and a soft, systolic murmur were noted. The results of the initial laboratory examinations are listed in Table 3. A greatly reduced serum iron concentration (9 γ/100 ml.) was present. The serum copper concentration was normal (142γ/100 ml.). The serum protein concentration was moderately reduced (alb., 3.8; glob., 1.2 gm/100 ml.). The anemia was microcytic (mean corpuscular volume, 57μ^3) and hypochromic (mean corpuscular hemoglobin concentration, 24%). No source of blood loss was found. Roentgenographic studies of the upper and lower gastrointestinal tract were normal. The first stool examined showed a trace of occult blood but two subsequent specimens were negative. Urinalysis was normal.

The patient was treated with an intramuscular iron preparation in an amount equivalent to 300 mg. of elemental iron.§ There was a prompt reticulocyte response and the hemoglobin concentration increased 3.6 gm. one week after therapy was initiated. The patient was discharged on Sept. 30, 1955.

A follow-up visit on Feb. 2, 1956, revealed a normal child. The laboratory findings were all within the range of normal (Table 3).

CASE 4.—A 9-month-old white boy was referred to this laboratory on July 1, 1955. Although "swelling of the eyelids" had been present during the preceding four months and an "anemia" had been suspected for two months, the mother had considered the infant well. He was the product of a full-term, first pregnancy. The birth weight was 4170 gm. (9 lb. 3 oz.). The pregnancy, labor, and delivery were uncomplicated. His development was regarded as normal. One upper respiratory infection and one episode of mild diarrhea were the only past illnesses. There was no history of symptoms suggestive of urinary-tract disorder. The infant had been breast fed for two and one-half months then changed to whole milk feedings. Strained foods had been added at about 3 months of age and chopped foods at 6 months of age. However, owing to "food allergies" the solid food intake was severely restricted. From about 6 months of age until seen by us, he had been receiving 32 to 40 oz. of milk per day.

On physical examination the baby was noted to be obese and irritable. There was moderate pallor and periorbital edema. There were no other significant physical findings. The initial laboratory findings are tabulated on the first line of Table 4. These showed marked hypoferremia (serum iron, 11γ/100 ml.) and hypocupremia (serum copper, 68γ/100 ml.). The mean corpuscular volume was 60μ^3 and the mean corpuscular hemoglobin concentration 24%. A test of the stool was positive for occult blood. Urinalysis was negative.

The initial treatment consisted of intramuscular injections on July 1 and 5, of 2 cc. of an iron dextran complex, each equivalent to 100 mg. of elemental iron.‖ In addition, five drops of a 1% solution of copper sulfate twice a day was prescribed. Initially, the diet was not altered. Within a week, on July 8, there was marked improvement in the serum iron and copper concentrations and the hemoglobin increased 2 gm. per 100 ml. (Table 4). The serum protein concentration did not improve. Meat-Base Formula in place of whole milk was prescribed. Two weeks later the hemoglobin had risen to 10.1 gm/100 ml., but the hypoferremia and hypocupremia had returned and the serum

§ Imferon, courtesy of Benger Laboratories, Holmes Chapel, Cheshire, England.

‖ Imferon, courtesy of Benger Laboratories, Holmes Chapel, Cheshire, England.

TABLE 4.—*Summary of Hematologic Data,* Case 4*

Date	Hb., Gm/100 Ml.	RBC, 10^6/Mm.^{3}	S. I., γ/100 Ml.	T. I. B. C., γ/100 Ml.	% Sat.	S. C., γ/100 Ml.	E. P., γ/100 Ml.	Serum Proteins, Gm/100 Ml.		Remarks
								Alb.	Glob.	
7- 1-55	6.8	4.66	11			68	214	2.0	0.8	Swelling of eyelid previous 4 mo.; 200 mg. Fe. I. M.; $CuSO_4$ 1% solution orally
7- 8-55	8.8		92			108	183	1.7	0.8	Meat-Base Formula prescribed
7-22-55	10.1	4.01	23			60	123	1.6	0.8	Milk intake restricted
8- 9-55	10.7	4.75	34	414	8	60	65	2.7	0.9	
10-18-55	12.7	4.84	68			117	31	3.2	0.9	
3- 7-56	13.8	5.38	122			156	11	4.7	1.5	

*For explanation of headings, see footnote to Table 1.

protein concentration remained unchanged. It was determined that the mother had not given the Meat-Base Formula and only sporadically administered the copper sulfate solution. Admission to the hospital was strongly recommended. The mother refused this advice but agreed to limit the infant's milk intake and to give the Meat-Base Formula. The subsequent course was one of very gradual improvement. The hemoglobin, serum iron, and serum copper concentrations were normal when the patient was seen three months later (Oct. 18, 1955). At that time the serum proteins had risen to 4.1 gm/100 ml. Table 4 lists the changes in the erythrocyte and hemoglobin concentrations and the blood chemistry values during therapy. When the patient was last studied, March 7, 1956, no abnormalities were present.

CASE 5.—A 2½-year-old white girl was referred to our laboratory on Jan. 3, 1956, with a history of anemia and "puffiness of the eyes" for the preceding 8 months.¶ She was the product of a first pregnancy. The pregnancy was of approximately 34 weeks' duration and was complicated by moderately severe toxemia during the month prior to delivery. The child was delivered with the aid of low forceps after a seven-hour labor. The birth weight was 2340 gm. (5 lb. 3 oz.) Her development was normal. An ear infection with mild diarrhea, at 5 months of age, was the only past illness. The child had been found to be allergic to dust, feathers, and wool. Tests for food allergies had not been performed. Symptoms of urinary-tract disease had not been noted.

¶Dr. John Diamond, of the Southern California Permanente Medical Group, referred this patient to us and assisted in the follow-up observations

Breast feeding was discontinued after two weeks, because of the mother's "nervousness." An evaporated milk formula was given until the infant was 5 months of age. Subsequently, homogenized milk was the principal item of her diet. Up to 48 oz. per day were ingested. Solid foods were added to the diet after the fourth month but were refused or given in minimal quantities. Meat alone was refused, and a prepared meat-vegetable combination was taken only if added to other bottle feedings. Six months before being sent to our laboratory, the infant was found by the physicians in charge to have a hypochromic anemia and marked reduction in her serum protein concentration. At that time the blood urea nitrogen was 10.5 mg/100 ml.; the serum cholesterol, 145 mg/100 ml.; the icterus index, 3.6, and the cephalin flocculation negative at 24 hours, 1+ at 48 hours.

In an attempt to correct the hypoproteinemia, dehydrated milk, equivalent to approximately 15 to 25 gm. of milk protein, was added to the child's milk feedings per day. An iron preparation also was prescribed. Administration of the iron preparation was sporadic because the mother did not want to "upset her child." The anemia persisted and the serum protein concentration remained unchanged. The patient was then referred to us on Jan. 3, 1956.

The physical findings were normal except for periorbital edema and moderate pallor. Laboratory findings on admission are tabulated on the first line of Table 5. These showed marked hypoferremia (serum iron, 10γ/100 ml.), hypocupremia (serum copper, 24γ/100 ml.) and hypoproteinemia (alb., 2.1; glob., 0.8 gm/100 ml.). A microcytic (mean

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TABLE 5.—*Summary of Hematologic Data,* Case 5*

Date	Hb., Gm./100 Ml.	RBC, 10^6/Mm.^{3}	S. I., γ/100 Ml.	T. I. B. C., γ/100 Ml.	% Sat.	S. C., γ/100 Ml.	E. P., γ/100 Ml.	Serum Proteins, Gm/100 Ml.		Remarks
								Alb.	Glob.	
6-24-55	7.9							1.9	1.5	Puffiness of eyes; 15-25 gm. milk protein added to diet
9-24-55	8.8							2.0	1.4	
12-22-55	9.2	4.17						1.8	1.3	
1- 3-56	7.5	4.40	10	159	5.7	24	213	2.1	0.8	First visit to our laboratory
1-23-56	9.6	5.06	22	287	7.7	31	165	2.0	0.8	
2- 4-56	10.0	4.21	23	223	10	48	164	2.4	0.8	
3-17-56	10.2	4.38	31	191	16	57	82	2.6	1.1	Hospitalized; high-protein diet; milk restricted
3-27-56	10.3	4.88	90	455	20	91	80	3.8	1.4	
4- 3-56	11.4	4.66	49	449	11	117	91	4.1	1.6	

* For explanation of headings, see footnote to Table 1.

corpuscular volume, 72μ^3), hypochromic (mean corpuscular hemoglobin concentration, 24%) anemia was also present. Urinalysis was negative.

Meat-Base Formula was prescribed, but later, on questioning the mother, it was apparent only a minimal amount had been given, probably less than 1 oz. per day. In addition, ferrous sulfate was again advised but, as before, not given.

During this home management regimen, there were no changes in her laboratory findings, when checked on Feb. 4 and March 17. The child was then hospitalized and placed on a carefully observed high-protein diet. Milk was restricted to 1 pt. or less per day. On April 3, after two weeks on this diet, there was a marked increase in the serum protein concentration paralleled by a rise in the serum iron-binding capacity. The serum copper concentration had increased to normal. The hemoglobin concentration rose to 11.4 gm/100 ml. She was discharged from the hospital with instructions to the mother to continue the hospital diet. A follow-up study, five weeks after dis-

TABLE 6.—*Serum Protein Concentrations in Five Infants with Hypocupremia and Iron Deficiency Anemia Before and After Treatment*

Case	Initial Observations					Final Observations				
	Serum Albumin Gm/100 Ml.	Serum Globulin, Gm/100 Ml.				Serum Albumin, Gm/100 Ml.	Serum Globulin, Gm/100 Ml.			
		α_1	α_2	β	γ		α_1	α_2	β	γ
1*	2.0	0.7		0.7	0.0	4.1	0.1	0.6	1.2	0.8
2	2.1	0.5	0.5	0.8	0.2	3.5	0.3	0.5	0.7	0.6
3	1.0	0.3	0.5	0.7	0.4	3.2	0.3	0.9	1.3	0.9
4	0.8	0.2	0.8	0.7	0.3	4.0	0.2	0.7	1.0	0.4
5	0.8	0.3	0.8	0.8	0.3	2.4	0.3	0.8	1.0	0.6

* The initial observations for Case 1 were obtained with the use of a salt fractionation method at the Hollywood Presbyterian Hospital laboratory.

charge from the hospital, showed the hemoglobin concentration to be 11.4 gm/100 ml.; the serum iron concentration had dropped to 36γ/100 ml., the serum copper concentration was normal (114 γ/100 ml.) and the serum protein concentration again moderately decreased (alb., 3.6; glob., 1.5 gm/100 ml.)

Summary of Cases

Case reports of five patients with hypocupremia and hypoproteinemia associated with iron deficiency anemia have been presented. At the time of diagnosis the patients ranged in age from 6½ to 9 months, with the exception of Case 5, who was 2½ years old. All had in common a mild degree of edema and pallor. There were no other significant physical findings. All had moderate anemia (hemoglobin, 5.2-7.5 gm/100 ml.), microcytosis (mean corpuscular volume, 60-74μ^3), and profound reduction in the concentrations of serum iron (4-18 γ/100 ml.) and serum copper (24-68 γ/100 ml.). The total serum iron-binding capacity was not increased (159-368 γ/100 ml.), but the per cent saturation was greatly reduced (1.3%-5%). The serum protein concentration was reduced in all cases (2.7-3.8 gm/100 ml.). There was no laboratory evidence of renal or hepatic dysfunction. In all instances correction of these deficiencies was achieved with dietary changes. Recurrences of the syndrome have not been noted.

Except for the initial observation on Case 1, the serum proteins of all patients were studied with the use of filter-paper electrophoresis. The results at the time of initial observations and following therapy are summarized in Table 6. Comparison of the electrophoretic values for albumin concentrations with those obtained by the salt fractionation method, listed in tables 1-5, reveals a greater reduction in the albumin fraction. Table 6 also shows, on comparison of the initial observations with the final observations, marked reduction in the serum gamma globulin concentrations. However, changes in the alpha$_1$, alpha$_2$, and possibly the beta fractions are not distinctly evident.

Comment

The copper method employed in this study and in that of normal infants * measures the total serum (plasma) copper. With this method applied to 34 normal adult men, a mean value of 92 γ/100 ml. of serum with a standard deviation of ±21 was found; 95% of the values ranged from 57 to 134 γ/100 ml. In a group of 21 carefully selected normal adult women, a mean serum value of 104 ±13 γ/100 ml., with a range of 85-128 γ/100 ml. was found. In the study of normal infants the mean value at 4 months of age was 100 γ/100 ml., with numerous low values (below 80 γ/100 ml.) observed prior to that time. In infants over 4 months of age the upward slope of the mean curve reached approximately 140 γ/100 ml. at 8 months of age, where it remained throughout infancy; the spread of values was from 85 to 200γ. In general, it appears that from 4 months of age throughout infancy the normal serum copper concentration is within a maximum range of 80-180γ.† In 17 older children, 3 to 10 years of age, a mean value of 131 with a range of 99-188 γ/100 ml. was found.

The initial serum copper concentrations, in the patients presented in this report, were, according to increasing values, 24, 28, 37, 43, and 68 γ/100 ml. The ages in months corresponding to these values were 30, 7½, 6½, 9, and 9, respectively. All of these infants also had a moderate degree of iron deficiency anemia. This, ordinarily, is associated with greater than normal copper concentrations. Bush [20] reported plasma copper concentration in 24 cases of iron deficiency anemia in infants; he found a mean value of 168 γ/100 ml. with a standard deviation of ±37. It can be concluded, therefore, first, that each of the infants studied by us had an initial serum copper concentration below the lowest limits of normal for their age, and, second, these

* Reference 4; Sturgeon, P., unpublished observations.

† Sturgeon, P., unpublished observations.

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values deviated in a direction from normal opposite to that associated with uncomplicated iron deficiency anemia.

The profound hypocupremia observed in these five cases of iron deficiency anemia indicates that nutritional copper deficiency occurs in the human. Although studies of total body copper or tissue copper have not been performed in our cases, the probability that the hypocupremia represents an actual depletion of total body copper is suggested by several facts. It has been established that in experimentally produced and naturally occurring copper deficiency in animals there is an associated hypocupremia.# The rapid increase in serum copper concentration observed in cases 3 and 4 after oral administration of copper, and after "correction" of the diet in cases 1, 2, and 5 would indicate that the observed hypocupremia was not a manifestation of failure to assimilate copper or to mobilize copper from stores or to retain copper. The occurrence of this condition in association with other evidences of malnutrition, including iron deficiency and hypoproteinemia, also suggests its nutritional origin. Furthermore the fact that it is observed during a period of rapid growth with the organism subsisting almost exclusively on milk, a food known to be an extremely poor source of copper,[18] is also consistent with the above conclusion. In contrast, in the hypocupremia associated with nephrosis, Wilson's disease, and sprue other mechanisms are more likely to account for the low serum copper concentration. In nephrosis, increased urinary excretion of copper in association with the proteinuria has been demonstrated.[6] In Wilson's disease there is a marked reduction or absence of circulating ceruloplasmin,[29] the alpha$_2$-globulin with which 96% of the plasma copper is bound,[20] and in addition there is increased urinary excretion of copper in association with aminoaciduria.[8] Only preliminary studies of copper metabolism in sprue have been reported, but it has been suggested that the low serum copper concentration in these cases is the result of impaired absorption of copper.[7] The patients described in this report showed none of the urinary, optic, or other clinical and laboratry abnormalities associated with these latter conditions. It seems apparent, therefore, despite the prevalent belief that nutritional copper deficiency is not observed in humans, that it does occur in infants.

The existence of naturally occurring copper deficiency in animals has been reported from many parts of the world. This subject has been reviewed by Marston.[27] A hypochromic anemia, changes in the coats of the animals, cachexia, decreased milk production and reproductive capacity develop in copper-deficient cattle. In addition to the above changes, a demyelinating disorder of the nervous system is associated with copper deficiency in sheep. The various disorders are readily corrected by oral or parenteral administration of copper.

Copper deficiency in swine* and in rodents[26] can be produced experimentally by feeding copper-deficient diets and can be corrected by addition of copper to the rations. In these animals a microcytic, hypochromic anemia refractory to iron therapy is observed. In the dog copper deficiency also may be induced experimentally; however, the associated anemia is normocytic and normochromic.[30] In the hypocupremic states in animals, alterations in serum protien have not been reported and the associated anemias are corrected upon the administration of copper without additional iron. In contrast, in our cases the iron deficiency anemia seems to be fundamentally the result of a total depletion of iron rather than a failure, secondary to the copper deficiency, to utilize it for hemoglobin formation. Our studies are not adequate, however, to demonstrate whether the anemia and/or hypoproteinemia can be corrected independently of the hypocupremia in any or all such cases.

The interrelationship of the several de-

References 16, 26, and 27.

* References 16 and 17.

ficiencies is not clear from the presently available data. On the one hand, it is plausible that this syndrome represents coexisting, totally independent, multiple deficiencies. On the other hand, the possibility of one of the deficiencies being more or less fundamental to the others is not untenable. The answer to these considerations will have to await further investigation.

Etiologically, it appears quite likely that nutritional inadequacy is of primary importance in the pathogenesis of this hypocupremic syndrome. However, the possibility that factors such as reduced body copper content at birth and rapidity of growth may play a part in the development of this syndrome, must also be considered. These factors are of importance in the pathogenesis of iron deficiency anemias, especially in infants from 6 to 18 months of age.[31] From the nutritional standpoint it is also important to consider a possible role played by modern food processing in the apparent relatively recent appearance of this syndrome. Preliminary investigations have revealed that one of the objectives in milk processing is to prevent access of copper to the milk at any stage of production. This, apparently, is desirable because copper has a deleterious effect on the storage and flavor of milk.[32] The probability must also be acknowledged, however, that this syndrome has always been in existence, but only because of the recent advent of techniques for the measurements of copper concentrations applicable to clinical material, and especially infants, has the condition been brought to light.

The role of blood loss deserves consideration as a possible etiology in two of these cases. In Case 3 the reoccurrence of profound iron deficiency anemia following its earlier correction by blood transfusion indicates that blood loss did occur in that infant. At that time, however, there was neither hypoproteinemia nor hypocupremia, suggesting that the reoccurrence of anemia developed independently of the original syndrome. Case 4 also had positive tests for occult blood on two occasions. Furthermore, it is of interest to note in the report of De Vries [33] on copper studies in adults there were eight cases with significant degrees of hypocupremia observed in persons who had iron deficiency anemia presumably secondary to chronic blood loss.

Summary and Conclusions

Although it has been generally accepted that the serum copper concentration is normal or elevated in iron-deficiency anemia, a review of the literature reveals that some cases of iron-deficiency anemia have been described, which were associated with a degree of hypocupremia.

Preliminary observations in this laboratory have revealed five cases of iron deficiency anemia associated with marked reduction in serum copper concentration.

In association with the hypocupremia and iron-deficiency anemia were findings of decreased serum iron concentration, decreased per cent saturation of the iron-binding protein, and increased free erythrocyte protoporphyrin concentration. The total serum iron-binding capacity was not increased.

In addition, the five cases had markedly diminished serum protein concentrations. Peripheral edema was a presenting finding in each case.

Of probable etiologic importance to all cases was their subsistence on a milk diet to the exclusion of practically all other foods.

Preliminary therapeutic tests indicate that the various deficiencies can be corrected simultaneously with an improved diet and that, independent of the other deficiencies, the hypocupremia can be corrected with the oral administration of copper sulfate.

Addendum

Subsequent to the completion of this study, M. E. Lahey and W. K. Schubert of the Children's Hospital, Cincinnati, reported on May 8, 1956, to the Society for Pediatric Research their observation on

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"new deficiency syndromes occurring in infancy." They described five infants from 6 to 15 months of age with serum copper concentrations of 37 to 61 γ/100 ml. who also exhibited hypoproteinemia and iron deficiency anemia. They found no excessive urinary loss of protein, amino acids, iron, or copper. Appropriate dietary therapy restored their patients to normality.

Through personal communication it has also come to our attention that R. A. Ulstrom, N. J. Smith, and E. M. Heinlich, of the University of California School of Medicine at Los Angeles, have had under observation instances of hypoproteinemia in infants. As in our cases and those of Lahey and Schubert, reduced serum copper concentrations were observed. However, their material, to be reported in this journal, involves younger infants whose diets were considered adequate, and dietary therapy did not restore their patients to normality.

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The Management of Inguinal Hernia in Infancy and Early Childhood

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Inguinal hernia is the commonest congenital lesion for which infants and children have been admitted to the Children's Memorial Hospital since its opening, in 1948. Its incidence exceeds three to one the next commonest abdominal lesion, which is congenital hypertrophic pyloric stenosis. Among 24,000 admissions to the hospital from March, 1948, to July 1, 1955, 521 admissions have been for inguinal hernia, in contrast to 176 for congenital hypertrophic pyloric stenosis.

It is our purpose to summarize some of the pertinent clinical aspects of this common lesion and our experience in its management. The material consists of a consecutive series of 298 inguinal hernias seen in 240 infants and small children on the surgical service of one of us (C. W. McL.) and which have been critically reviewed.

Embryology

The processus vaginalis appears as a sac-like pocket of peritoneum on each side of the ventral abdominal wall during the third month of fetal life. By the sixth fetal month, the testis comes to lie near these peritoneal processes but maintains a retroperitoneal relationship. Without change in position of the testes or their relationship to the peritoneum, the processus vaginalis elongates through the inguinal canal into the scrotum. The proximal portion of the process, which lies within the inguinal canal, is referred to as the funicular portion, and the distal portion, which later forms the tunica vaginalis of the testes, is referred to as the testicular portion.

The gubernaculum testis extends dorsal to the peritoneum from the caudal pole of each testis to the scrotal wall. Between the seventh and ninth fetal months and coincident with the shortening of the gubernaculum the testes descend rapidly behind the peritoneum to the depths of the scrotum. Here each is covered with a reflected fold of the processus vaginalis, known as the tunica vaginalis of the testicle.

Within a few months after birth the narrow canal which connects the testicle with the abdominal cavity becomes obliterated and the lining epithelium absorbed. When the normal developmental pattern has been completed, the testicular portion of the processus vaginalis forms the tunica vaginalis and is a closed sac. The funicular portion is obliterated, and there is no peritoneal tissue between the two serous cavities.

When the funicular portion of the processus vaginalis does not become obliterated, a potential hernia exists, and when in addition the testicular portion or tunica vaginalis of the testis remains patent, a complete funicular hernia is present. Herzfeld [1] states that the partial funicular type (incomplete hernia) accounts for 95% of the cases, while complete funicular hernias were noted in only 5% of his cases. In our reported series 68% were of the partial funicular type and 32% complete (Table 1).

Sex Incidence and Age

Inguinal hernias occur in males and females in a ratio of approximately 10:1. In our series there were 217 males (90.4%)

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TABLE 1.—*Inguinal Hernia in Infancy and Childhood—Series of 240 Patients with 298 Hernias*

	No. Cases	Per Cent
Boys	217	90.4
Girls	23	9.6
Presenting hernia (right)	161	67
Presenting hernia (left)	50	21
Presenting hernia (bilateral)	29	12
Incomplete inguinal hernia	202	68
Complete inguinal hernia	96	32

and 23 females (9.6%). This is as reported by Larsen,[2] Herzfeld,[1] Gross,[3] and Watson.[4]

It is common for the hernia to be noted at birth or during the first few months of life when straining or crying makes its presence evident. Some of the largest hernias have been seen in small infants and three in premature babies.

The ages at which the hernias were first noted in this consecutive group of 240 small patients are listed in Table 2. It is significant that 58.7% were evident by the end of the first year of life and within the first three years 78.1% were admitted for treatment. Although this analysis covered the pediatric age group from birth through 12 years of age, in only 21.9% of the patients admitted did the hernia make its appearance after the third year.

Bilateral Hernia

In our series of 240 patients, 29, or 12%, presented on initial examination with bilateral hernia. In 20 of the 29 cases the hernias appeared simultaneously on the two sides, while in 9 instances the second hernia appeared before the child came to surgery or was unknown to the mother until the initial surgical examination.

TABLE 2.—*Inguinal Hernia in Infancy and Childhood—Age at Which Hernia First Noted in Series of 240 Patients*

Age When Hernia Noted	No. Cases	Per Cent
Birth-1 mo.	37	15.3
1-3 mo.	39	16.5
3-6 mo.	45	18.6
6 mo.-1 yr.	20	8.3
1-3 yr.	46	19.4
3-6 yr.	38	15.7
6-12 yr.	15	6.2
	240	100.0

In the course of this study a total of 16 patients returned with a second hernia, which made its appearance on the opposite side after primary repair of one side. In 12 of these 16 cases the primary hernia was on the right side and in 4 on the left side. The interval between the initial unilateral repair and the appearance of a hernia on the opposite side was as follows: one year or less, seven cases; one to two years, one case; two to four years, five cases; over four years, three cases.

Because of this unhappy experience, which is most distressing to all, we have recently recommended and practiced routine exploration of the opposite side in all unilateral hernias under the age of 3 years, providing the general condition of the infant was satisfactory.

TABLE 3.—*Inguinal Hernia in Infancy—Results of Blind Exploration of Opposite Side with Single Primary Hernia in Series of Forty-Eight Infants from Birth to Three Years of Age*

	No. Cases	Per Cent
Primary hernia—right	37	
Hernia found on left	17	46
Hernia not found on left	20	54
Primary hernia—left	11	
Hernia found on right	8	73
Hernia not found on right	3	27

The results of routine exploration of the opposite side in a series of 48 infants under the age of 3 years is listed in Table 3. While the series is small, it is noteworthy that in 25 instances, or 52% of the group, a second definite hernia was found and corrected at the primary operation, preventing the necessity of a subsequent procedure. It is to be emphasized that in none of these cases could a hernia be demonstrated on the blindly explored side prior to surgery. Our figures support the findings of Swenson,* who observed that there was a higher incidence of a second hernia found when the initial hernia was left-sided, as would be anticipated in view of the known greater frequency of right-sided lesions. We firmly

* Swenson, O.: Personal communication to the authors.

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believe that in experienced hands exploration of the contralateral side is a sound surgical practice if the infant is under 3 years of age and in good general condition.

Diagnosis

The diagnosis in itself is not usually difficult, although the small size of the inguinal canal does not lend itself to digital examination as in the adult. A definite diagnosis is substantiated when an abnormal swelling is noted in the inguinal region during crying or straining which readily disappears with digital pressure over the canal.

Occasionally the hernia cannot be seen to come down or be apparent with crying during repeated examinations. In such cases the history given by the mother of a recurrent swelling in the inguinal region which disappears spontaneously is of great value. Further information is obtained by gently compressing the cord structures in the canal beneath the examining finger and noting a thickening of the cord in contrast to the opposite side. We have in seven instances operated on children under such circumstances, and a definite hernial sac was found in every case.

There are, however, certain pitfalls in differential diagnosis of an inguinal mass. Transillumination as an aid in the differential diagnosis in infancy is hazardous and unreliable, since an inguinal hernia containing bowel may readily be confused with a hydrocele. Compression of a communicating hydrocele with a large opening may yield an effect similar to the reduction of a hernia, and, conversely, an incarcerated hernia in the infant on compression will not change in size, as is also true of a hydrocele of the cord. The general appearance of the infant and the history obtained from the mother are of great value in these confusing problems, and we believe one must explore a small number of these cases where the diagnosis is in doubt.

An undescended testis can be easily differentiated by its absence from the scrotum, and this diagnosis itself practically confirms the presence of a patent or potential hernia. Dillon [5] reports that in a series of 300 orchiopexies a potential or patent processus vaginalis was present in every instance. One of us (C. W. McL.) has observed only 2 cases in a series of 150 patients coming to surgery for undescended testes in which a patent sac was not present. In both instances the testis was completely intra-abdominal.

Suppurative inguinal adenitis may sometimes be extremely difficult to differentiate from an incarcerated inguinal hernia. Here one must depend much on the history obtained from the mother regarding duration of the mass, coupled with a careful search for a primary site of infection. We have on two occasions operated upon a suppurative inguinal adenitis with a preoperative diagnosis of incarcerated hernia. In both instances the mass was said to have been present less than eight hours. In reviewing the records of these two infants, the white blood cell count was moderately elevated in both, and it was noted that the skin over the mass assumed an abnormal pink blush with the soap-and-water preparation routinely used in surgery, suggesting this possibility.

Incarcerated Hernia

Incarceration is the common complication of hernia in this age group, whereas true strangulation rarely occurs. Herzfeld [1] reports the ratio of uncomplicated to incarcerated hernia to be about 10:1. In our series there were 26 infants admitted with incarcerated hernia, representing 10.8% of the group, but we have not as yet observed strangulation of bowel in an infant hernia.

Thorndike and Ferguson [6] found incarceration in 106 of 1740 inguinal hernias but only 5 cases of strangulation. MacLennan,[7] in an extensive review of 10,384 cases, found only 4 true strangulations.

The greatest incidence of incarceration occurs during the first 6 months of life, rapidly diminishing with growth, and to be quite rare after the age of 6 years.

In our series of 26 cases, 19, or 73%,

were males, and 7, or 27%, were females. This sex ratio is somewhat different from that usually reported, which states that 90% or more are seen in males. Our experience confirms that of other observers in that 69% of the group were under 3 months of age on admission and no cases were seen above 3 years of age (Table 4).

In five cases in this series the hernia reduced while anesthesia was being induced, but the operation was proceeded with in each instance. In those small patients in which reduction occurs after hospital admission prior to surgery, operation is always advised during the hospital stay, but a delay of two or three days is helpful to permit edema in the canal to subside.

TABLE 4.—*Incarcerated Inguinal Hernia in Infancy —Series of Twenty-Six Cases (Nineteen Boys and Seven Girls)*

Age on Admission	No. Cases	Per Cent
Birth-3 mo.	18	69
3-6 mo.	1	4
6 mo.-1 yr.	3	11.5
1 yr.-3 yr.	4	15.5
	26	100.0

In the 26 cases which were operated on as surgical emergencies, red but viable ileum was found in the sac in 16 cases, the cecum and appendix in 2 cases, and the tube and ovary in 8 instances. In one of the latter infants, the tube and ovary were gangrenous, necessitating removal, this being the only instance of nonviable tissue noted.

Surgical Repair of Inguinal Hernia

During the past 15 years we have recommended the immediate repair of all inguinal hernias regardless of age. While it must be accepted that herniorrhaphy in a child under the age of 3 months is a more difficult operation than in older children, familiarity with the infant anatomy, the use of fine instruments, and, above all, delicate handling of tissues make it a sound surgical procedure. We routinely employ open drop ether, and it is ideal in the hands of anesthetists accustomed to working with infants and small children. No premedication is given to children under 3 months of age. In those from 3 months to 2 years we employ atropine only in appropriate dosage. In older children, secobarbital (Seconal) administered by rectum with atropine hypodermically or atropine and morphine in appropriate dosage are given one hour before surgery.

In infants under 3 years of age a small transverse incision beginning at the anterosuperior spine of the ilium and projecting medially for a distance of 3 cm. in the lower abdominal crease is routinely employed. This incision with retraction gives very adequate exposure of the inguinal area and permits adequate visualization of the entire inguinal canal. The sac is carefully picked up with very fine forceps, lying as it invariably does on top of the cord structures. If the sac is incomplete, the index finger is introduced into the tip of it, and with extremely careful gauze dissection all adherent structures are freed until its neck is clearly delineated at a high level. The sac is then ligated and permitted to retract upward but not tacked to the adjacent tissue at this level.

If the hernias are found to be complete with the testis in the lower portion, we carefully free the sac from the adjacent cord and vessels, dissecting it upward to the internal ring and carry out a similar high ligation. This is always a more difficult procedure, since the sac in a complete hernia always seems abnormally thin, and even greater care must be exercised to prevent its being badly torn, which complicates a good ligation of its neck. The distal portion of the sac about the testis is removed as completely as possible. That portion around the testis cannot be removed in its entirety but is trimmed away, and usually a single stitch everts that which remains to prevent the subsequent development of a testicular hydrocele. It is extremely important that the testis be carefully replaced in its natural bed deep in the scrotum after correction of a complete hernia, or one may subsequently find that it lies in an abnormally high position and is subjected to trauma.

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While repair of the inguinal canal is considered unnecessary by many, we favor closing this as in the Fergusson technique, with two or three interrupted sutures. One must always leave a very adequate space for the emergence of the cord. The external oblique fascia is reapposed and the skin closed with a fine subcuticular suture. In our cases we have routinely employed very fine absorbable surgical suture (gut) throughout including the skin and have been eminently satisfied with its use in this infant group.

No dressings are employed, but the incisions are covered with a small coating of collodion or, more recently, sprayed with vibesate (Aeroplast). When this is almost dry the wound is dusted with talc and the baby returned to its crib.

The classical inguinal incision is entirely adequate but has the disadvantage in children under 3 years of being more exposed to soiling and demands working in a deeper wound because of the almost constantly present suprapubic fat pad in these small patients. In children above the age of 3 we employ the inguinal incision, but the Fergusson technique is entirely satisfactory for repair in the entire pediatric age group.

We have used no restraint on return from surgery, and we put these small patients back on regular diet as soon as they will accept food. Postoperative medication for pain is practically never necessary in those under 3 or 4 years of age. Wound infection is conspicuous by its absence, and the patients may be dismissed any time after the second day. We do not remove the protective covering from the incision until the infant is reexamined—an average of one week after surgery. If it has not separated by this time it may be easily removed and the underlying wound found healed. On dismissal of the patient from the hospital the mother is instructed that the infant or child may be normally bathed in the tub each day.

In this series of 298 hernias we have encountered 20 with associated hydroceles and 5 with hydroceles of the testis. Eleven patients in this group had undescended testes cared for at the time of herniorrhaphy.

Comment

There is general agreement among those particularly interested in the surgery of infancy and childhood that all inguinal hernias should be repaired as soon as the diagnosis is established. Unfortunately, there are still many who advise parents that operation should be delayed until the child reaches the age of 3 or 4 years. Admittedly, operation may be a little easier with the older child, but unfortunately, as noted, the majority of these little patients developed their hernias during the first two or three years of life, and its management becomes a real problem for the mother if surgery be delayed. There is practically no support for the idea that an established hernia in an infant will obliterate itself spontaneously. Conservative means using trusses of any known type have been extremely unsatisfactory in our hands, and it is very unusual to find a mother capable of maintaining constant reduction of a hernia by such means. Furthermore, these which do become complicated by incarceration occur almost entirely in this age group and demand immediate repair. Since the small infant tolerates a well-given anesthesia very well and the repair is entirely feasible in capable hands, we see no reason for withholding surgical correction from all cases regardless of age.

TABLE 5.—*Inguinal Hernia in Infancy and Childhood—Age of Patients Coming to Surgery in Series of 240 Patients*

Age	No. Cases	Per Cent
Birth-1 mo.	11	4.6
1-3 mo.	60	25
3-6 mo.	10	4.2
6 mo.-1 yr.	15	6.2
1-3 yr.	54	22.5
3-6 yr.	57	23.8
6-12 yr.	33	13.7
	240	100.0

The age of the infants and children in this series coming to surgery is listed in

Table 5. Over 40% of the group were operated upon during the first year of life, and 62.5% came to surgery before the third year of life. There has been no mortality in this group and no serious complications. One recurrence has been noted to date. This infant was operated upon at the age of 3 years, with bilateral communicating hydrocele. He was seen three years later with a definite left inguinal hernia which on repair was found to have a 2 cm. sac. We have considered that this case must be listed as a recurrence.

Summary

A series of 298 hernias occurring in 240 infants and young children is critically reviewed.

Inguinal hernia is the commonest congenital lesion for which infants and children have been admitted to this hospital.

In almost 60% of the instances the hernia was noted in this series of cases before the first year of life.

Seventy-three per cent of the complicated hernias in our series occurred in infants under 6 months of age.

The surgical repair of inguinal hernias in infancy should be undertaken as soon as the diagnosis is made and is a safe and practical procedure in this age group.

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Measles Meningoencephalitis

A Summary of Twenty-Four Cases Treated at Grasslands over a Ten-Year Period

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Introduction

The central nervous system complications of measles were recognized as early as 1872, when Westphal first used the term acute disseminated encephalomyelitis. Measles encephalitis as a clinical entity was very adequately described by Neal and Appelbaum, in 1927,[4] and by Musser and Hauser, in 1928.[7] An increasing interest in the subject of measles encephalitis in recent years has stimulated a search for some form of specific therapy. In 1952, corticotropin (ACTH) was first tried in a series of patients by Appelbaum, at Willard Parker.* Gamma-globulin has subsequently been tried in the treatment of measles encephalitis; however, experience has indicated that it is not an effective therapeutic agent.[6]

The purpose of this presentation is to summarize the experience over a 10-year period with measles meningoencephalitis occurring in the pediatric age group at Grasslands Hospital. There have been 24 diagnosed cases of measles meningoencephalitis treated here since 1945, and I should like to present an analysis of clinical features, laboratory findings, sequelae, recent advances in therapy, and other statistical data referable to age, race, and sex.

The average age group in this series of patients was 5-6 years. The youngest patient was 17 months of age, and the oldest, 11 years. Of the 24 cases, one was a Negro child and the remainder white. There were 10 girls in this series and 14 boys.

The encephalitic manifestations were observed to occur at any time during the clinical course of measles. One of the cases occurred on the first day of the prodromal phase of the illness, well before the appearance of the exanthem.[3] Six of the patients developed encephalitis, either concomitant with the appearance of the rash or within a 24-hour period after the onset of the rash. Sixteen patients developed encephalitic symptoms and signs either during the period of maximal intensity of the morbilliform eruption or after the eruption had begun to fade.[5] Three of the patients developed encephalitis as late as eight days after onset of prodromal symptoms.

The encephalitic symptoms and signs were varied. By far the commonest presenting manifestation was some alteration in sensorium. Every patient showed either lethargy, somnolence, stupor, or coma at some time during the illness. Approximately one-half of the patients were obtunded to the stage of stupor or coma. Five of these patients were admitted in profound coma, lasting as short a time as 24 hours in one instance and as long as 10 weeks in another.[5] In approximately one-half of the patients, adventitious movements of some sort occurred. Irregular, jerky, clonic, convulsive movements were noted with associated coarse tremors. In several of the patients a tonic phase of the convulsive seizure also occurred. In 8 of the 24 cases a resurgence of fever occurred at the onset of encephalitis. The combination of lethargy or somnolence, for example, with a sudden unexplained rise in temperature was a very common early sign. Other inconstant symptoms noted at onset, or within 24 hours of onset, were combativeness, extreme irritability, disorientation with impairment of memory for recent past events, hallucina-

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* Personal communication to the author.

tions, headache, eye pain, photophobia, slurred speech, and urinary incontinence or retention. In one patient urinary retention was the most important initial problem.

The clinical course of measles encephalitis in these patients was extremely variable. One patient who was comatose on admission remained in such a state for a period of 3 weeks; another child remained profoundly comatose for 10 weeks before even responding to painful stimuli. In both cases, aspiration pneumonia and atelectasis were constant problems, and bronchoscopic aspirations, followed by tracheotomy, were life-saving procedures. In the child who was comatose for 10 weeks, occasional convulsive seizures greatly complicated the aspiration problem. Urinary retention and persistent urinary tract infection were also complications in both cases. In each child, after the period of coma and stupor the clinical picture was characterized by involuntary movements of the extremities, combativeness, and irrational behavior which persisted as the sensorium cleared.

The onset of measles meningoencephalitis and its course may also be fulminating. One child was admitted with a 12-hour history of somnolence progressing to coma. He was admitted to the hospital in a moribund state, breathing at the rate of two to three respirations per minute, and in spite of a tracheotomy the patient died one hour after admission. In this child there was never any clinical evidence of measles. The diagnosis was made by autopsy findings of typical measles giant cells in lymphoid tissue, a giant-cell pneumonia, and an early lymphocytic infiltration of the meninges and perivascular spaces.

On the other hand, the onset and course of the disease may be very slow and insidious. In one child, there was gradual onset of a Parkinson type of clinical picture eight days after the first prodromal symptom. There was barely any residual evidence of a rash in this patient.[1] The neurological picture in this patient was characterized by a mask-like facies, marked salivation and difficulty in swallowing, cogwheel rigidity, a tremor at rest, aphonia, and a typical catatonic "waxy flexibility." This child over a two-week period gradually progressed into a completely responsive, alert, and asymptomatic picture and was discharged without any evidence of neurological sequelae.

In contrast to the severity of the cases just discussed, we had one child who presented in a very lethargic state which persisted for three days with several episodes of subnormal temperature. He then promptly reverted to his normal active behavior and was discharged asymptomatic. This child remained oriented and rational during his entire illness and never had a convulsive seizure.

The spinal fluid findings in measles meningoencephalitis are variable.† This variability has been demonstrated in other series of cases as well.[2] In 3 of our series of 24 cases, the spinal fluid was entirely normal. There was no pleocytosis and no increase in protein or sugar. However, in two of these three patients the protein rose slightly during the course of the illness, but it always remained below the upper limits of normal. In four other cases the only abnormality encountered was a slightly elevated protein, with the spinal fluid remaining acellular.

One of the commonest findings in the spinal fluid was an increase in cells and protein. Approximately one-half of the patients showed these changes. Another interesting finding was an elevated spinal fluid sugar, noted in seven cases. The highest elevations were noted in children who had been convulsing prior to lumbar puncture. In one of such patients a value as high as 152 mg. per 100 cc. was found. However, several patients had spinal fluid sugars above 80 mg. per 100 cc. with a history of only progressive stupor or somnolence. We have no definite explanation for these findings.

Electroencephalograms were taken in six cases, either during the acute phase of the disease or sometime later. In every case

† References 4 and 5.

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high-amplitude slow wave activity was noted with or without a preponderant focus.[3] Sometimes the abnormal wave patterns persisted for several months before approaching normal.

The therapeutic approach to measles encephalitis had largely been supportive in nature prior to the introduction of corticotropin by Appelbaum, in 1952. However, with the recent concept of a possible allergic inflammatory response within the central nervous system to the measles virus, it was hoped that the use of cortisone or corticotropin might definitely inhibit this process. In view of the devastating nature of measles encephalitis, with a high incidence of sequelae, steroid therapy was considered justifiable.

In our series of 24 cases over a 10-year period, cortisone or corticotropin has been used in 7 instances. With this small series of cases it is not possible to draw any conclusions as to efficacy of this type of therapy. In view of the extreme variability of the clinical course of measles encephalitis it would seem very difficult to make any justifiable conclusions concerning the adequacy of steroid therapy even among larger series of patients.

In comparing our 7 cortisone-treated cases with 17 non-cortisone-treated cases, we are not impressed that the clinical course of the illness was altered. In both groups there are patients whose sensorium cleared in 24-48 hours and patients whose clinical course was extremely prolonged and severe, with coma persisting as long as 3 weeks in one instance and 10 weeks in another.

However, from the standpoint of preventing sequelae we are definitely more impressed that steroids are of some benefit. It must be noted, however, that the two series of patients are numerically quite different, our cortisone-treated children representing less than one-third of the total of 24. Therefore, we must look upon these observations only as impressions and not as definitive conclusions. In both groups sequelae have occurred, but it is gratifying to note that among the cortisone-treated patients they have been of less severity. Behavior problems and personality changes occurred in five of the seven cases, and in one of these five children febrile convulsions and enuresis have occurred. Two of the seven patients have escaped without sequelae.

On the other hand, among the non-cortisone-treated group some serious sequelae have been noted. One child died with the added complication of a giant-cell pneumonia; one has mental retardation with a partial right hemiparesis; one patient is mentally retarded with the problem of occasional convulsive seizures and severe athetosis of the right upper extremity, and a fourth child has a partial right hemiparesis with persistent enuresis. Five other children have had minor behavior disturbances and personality changes. Six children have had no sequelae according to the parents, and in two cases no follow-up has been available. It is interesting to note that in each group of patients, comprised of 7 and 17, respectively, approximately one-third have escaped without sequelae; however, in contrast, the severity of the complications in the latter group cannot be matched in the cortisone-treated group. The types of sequelae to measles encephalitis are very variable.[2]

When complications occur from measles encephalitis, by far the most frequently encountered are behavior disturbances and personality changes. Emotional instability occurs frequently. Irritability, nervousness, and lack of concentration in school are frequent complaints of the parents. Among the personality changes noted, we have one child who developed extreme hostility, resentment, and combativeness to such a degree that intensive therapy was necessary. Another child who was noticeably shy and withdrawn before her illness became very aggressive and outgoing even to strangers.

The next most frequently encountered complication is memory impairment for recent past events. In two children marked perseveration was associated with a disturbed memory.

We are unable to determine the exact frequency of mental retardation in these children, since psychometric studies were done in only a few. However, in three cases the retardation was obvious enough that further studies were not necessary. This would then represent the third most frequent type of complication.

An upper motor neuron type of paresis was the next most frequent sequela, with three children so afflicted. One of these three patients, as was previously noted, had severe athetosis.

Enuresis was noted to occur with a similar frequency. Three children were discharged with a long-standing enuretic problem, and in two of them urinary incontinence and repeated episodes of cystitis occurred.

Less frequently encountered complications are (1) convulsive seizures, (2) obesity, (3) transient dizziness, (4) transient reduction in visual acuity, and (5) persistent photophobia.

Summary

This paper summarizes the cases of measles encephalitis encountered in the pediatric age group during the last 10 years at Grasslands Hospital. Data referable to age, race, and sex incidences are summarized. The clinical aspects of measles encephalitis are described, and several interesting and unusual cases are included. Laboratory confirmation of the diagnosis is discussed. Newer concepts of more definitive therapy with steroid hormones and corticotropin are summarized. Types of sequelae encountered in measles encephalitis are discussed.

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Chemical Growth Dynamics of the Skeleton in the Immature Rat

I. Normal Chemical Composition and Growth

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Introduction

This publication relates to the chemical dynamics of skeletal growth in the normal immature male rat. Data presented are introductory to a combined chemical, radiological, and histological interpretation of skeletal growth in this animal from birth through 75 days of age.*

During this period of life in the rat, individual skeletal parts as well as the skeleton as a whole undergo continuous and often disproportionate changes in growth and development.† The following studies were undertaken to correlate chemical growth and turnover of the entire skeleton with serial changes in the chemical anatomy and linear growth of long bones.

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This study received support from the Mary H. K. Welsh Endowment Fund and the E. C. Fleischner Memorial Fund to the University of California, and Medical School Research Grant No. 150 (Raschen-Tiedemann Fund) of the University of California Medical Center.

* References 1 and 2.

† References 3-9.

Methods and Materials

Male Wistar rats were used throughout this study. Litters were arranged at birth to contain approximately 8 to 10 male animals. All animals were weaned at 21 days of age to a standard diet (Diet 1),‡ which contained 0.76% calcium and 0.44% phosphorus by wet weight, with a calcium-phosphorus ratio of 1.7. A constant source of distilled water was supplied.

Animals were killed at appropriate intervals between 1 and 75 days of age, for analysis of either total skeleton or long-bone aliquots.

In certain groups carrier-free radiocalcium (Ca^{45})§ was injected daily, intraperitoneally, in 10 divided doses prior to 21 days of age, in order to permit an associated analysis of total and differential skeletal uptake and turnover of calcium. In addition, a study of uptake and distribution of dietary calcium between 21 and 32 days of age was conducted by balance technique, utilizing Diet 1.

‡ This diet was developed by the Institute of Experimental Biology, University of California at Berkeley, Calif., where it has been used extensively in growth studies. Its composition is as follows:

Ground whole wheat	67.5%
Casein, Tech.	15.0%
Skim milk powder (D-free)	7.5%
NaCl	0.75%
$CaCO_3$	1.5%
Melted fat	0.75%
Fish oil (Vit. A-D concentrate)	1.0%
(Vit. A, 750 units/gm. fish oil)	
(Vit. D, 300 units/gm. fish oil)	
KI solution (450 mg. KI/liter)	300 cc.
in 180 lb. batch.	

§ Ca^{45} used in this study was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tenn. As obtained, the preparation contained a specific activity of more than 1000 mc/gm. in a concentration of more than 1 mc/ml. and the purity was greater than 99%. For use in these studies, the original acidic $Ca^{45}Cl_2$ solution was diluted with normal saline, and was sterilized. Individual animals received either 10 or 20 μc total which contained trace amounts of elemental calcium.

Methods used included those pertaining to the preparation of the skeleton and skeletal aliquots for analysis, and techniques of chemical analysis and radiocalcium determinations.

For analysis of the total skeleton, the bled, skinned, and eviscerated carcasses were sectioned, extracted with an alcohol-ether mixture, dried to a constant weight, and ashed. The ash was weighed, dissolved in nitric acid, and diluted with demineralized water to an appropriate volume. Long-bone samples were freed of muscle, fascia, and epiphyses and were grouped, crushed, and similarly treated through the drying stage. The extracted, dried samples were weighed and divided into two portions: one was dissolved directly in concentrated hydrochloric acid for nitrogen determination; the other was ashed and dissolved as for the total skeleton. Diet, stool, and urine specimens were dried, ashed, and dissolved in a similar manner.

The solutions of the ashed specimens were used for determinations of calcium, phosphorus, magnesium, sodium, potassium, and radiocalcium (Ca^{45}).

Calcium and magnesium were determined by modifications of the method of Sobel and Hanok [10] and Orange and Rhein [11] and phosphorus by a modification of the method of Fiske and Subbarrow.[12] Sodium and potassium were determined by flame photometry (Beckman), utilizing radiation buffers as described by West and co-workers [13] to minimize the effects of interfering ions. Nitrogen was determined by a modification of the micro-Kjeldahl method after Kabat and Mayer [14] and Kirk [15] and radiocalcium by a modification of the method of Comar.‖

Demineralized water was employed in all procedures and for cleansing all glassware.

Results

For analysis of chemical growth of the total skeleton, groups of animals were killed at 1, 21, 25, 32, 45, and 75 days of age. Data from these studies are presented in Table 1.

Since significant amounts of calcium and ash presumably are derived only from the skeleton of the bled, skinned, and eviscerated animal,¶ values for recoveries of these elements were designated as "skeletal." The source of magnesium, while also designated "skeletal" in this Table, is less precisely known.

Discrepancies between individual animals at any one age, with respect to body weight, skeletal ash weight, and total skeletal calcium and magnesium, were significantly reduced when these measurements were evaluated in terms of common units, i.e.,

‖ References 16 and 17.

¶ References 18-21.

TABLE 1.—*Chemical Growth of the Total Skeleton, One to Seventy-Five Days of Age*

Age (days) / No. of animals		1 20	21 11	25 6	32 8	45 6	75 6
Body Wt. (gm.)	Av.	5.8	26	27	60	85	190
	Range	5.0–6.5	17–28	25–30	50–68	80–93	168–214
Skeletal Ash. Wt. (gm.)	Av.	0.06	0.51	0.71		2.2	5.2
	Range	0.05–0.07	0.40–0.64	0.69–0.72		1.9–2.5	4.6–5.8
Ash Wt. mg/gm. body wt.	Av.	10	24	26		26	27
	Range	9.4–11.6	23–25	25–28		26–27	26–28
Skeletal Calcium							
Mg.	Av.	12	140	200	440	710	1710
	Range	10.8–14.1	112–199	201–203		676–742	1580–1890
Mg/gm. body wt.	Av.	2.1	6.6	7.5	7.3	8.3	8.9
	Range	1.9–2.4	6.4–6.9	7.3–7.9		7.3–9.3	8.0–9.1
Mg/gm. Ash	Av.	210	270	290		320	330
	Range	199–213	262–283	283–291		316–322	300–344
Skeletal Magnesium							
Mg.	Av.	1.1	8.0	13	15	31	76
	Range	1.0–1.2	5.4–9.5	12.1–13.5		29–33	65–84
Mg/gm. body wt.	Av.	0.19	0.32	0.47	0.25	0.38	0.40
	Range	0.18–0.21	0.31–0.33	0.43–0.53		0.35–0.42	0.33–0.48
Mg/gm. Ash	Av.	19	13	18		14	15
	Range	18–20	12.6–13.8	17.1–18.9		12.6–18.3	13.2–15.6
Ca^{45} Recovery, % *	Av.		99			94	93
	Range		91–112			89–96	82–98

* Ca^{45} data were obtained from only six animals at 21 days of age.

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when the ash content was related to body weight and when total skeletal calcium and magnesium were related to ash weight. The consistently high recovery of parenterally administered Ca^{45} is also shown.

Table 2 presents data obtained by analyses of long-bone aliquots from other groups of animals at 21, 45, and 75 days of age.

the average uptake and excretory loss of ingested calcium between 21 and 32 days of age in a group of eight animals. The skeletal calcium content of this group of animals at the onset of the study (21 days) was estimated from data presented in Table 1. All other values were determined directly by analysis.

TABLE 2.—*Chemical Anatomy of Long-Bone Aliquots at Twenty-One, Forty-Five, and Seventy-Five Days*

Age (days)	21		45		75	
No. animals	15		10		13	
	Av.	Range	Av.	Range	Av.	Range
Body Wt. (gm.)	26	17–36	100	80–120	190	167–215
Tibial diaphyseal length, mm.*	15.4	15.0–15.7	25	23.9–25.4	31	29.4–32.5
Long Bone						
Dry Wt/Ash Wt.	1.96	1.94–2.08	1.68	1.56–1.79	1.54	1.51–1.57
Dry Wt-Ash Wt. / Dry Wt.	0.49	0.46–0.52	0.40	0.36–0.48	0.35	0.34–0.36
Calcium						
Mg/gm. Dry†	170	162–177	210	190–223	230	222–239
Mg/gm. Ash†	330	317–346	360	340–374	350	313–369
Magnesium						
Mg/gm. Dry	5.9	4.2–6.7	6.2	4.9–6.7	6.1	5.1–6.8
Mg/gm. Ash	12.5	12.0–13.0	10.5	8.2–12.2	9.4	8.2–10.5
Phosphorus						
Mg/gm. Dry	98	97.3–101	110	106–112	120	103–126
Mg/gm. Ash	190	190–197	180	177–190	180	157–198
Sodium						
Mg/gm. Dry	4.5	4.4–4.7	5.1	4.7–5.5	4.6	4.0–5.6
Mg/gm. Ash	8.8	8.5–9.3	8.7	8.1–9.3	7.1	6.3–8.6
Potassium						
Mg/gm. Dry	3.4	2.8–4.1	1.5	1.2–2.0	0.8	0.65–0.88
Mg/gm. Ash	6.7	5.5–8.1	2.6	2.0–3.3	1.2	0.98–1.46
Nitrogen						
Mg/gm. Dry	63	61–66	53	47–60	49	42–56
Ca^{45} Recovery‡						
%/gm. Dry	35	32–35	21	18–26	10	14–18
%/mg/Ca	0.20	0.18–0.21	0.10	0.09–0.12	0.04	0.04–0.05

* Data pertaining to tibial length are presented in detail in a subsequent publication in this series (Reference 2).
† "Dry" and "ash" refer to the dry weight and ash weight of skeletal aliquots.
‡ Ca^{45} data were obtained from only six animals each at 45 days and 75 days.

Averages presented in Table 1 and Table 2 have, in general, been rounded off to two significant figures. All individual elements have been reported in relation to extracted dry weight and ash weight of bone aliquots, with the exception of nitrogen which is related only to dry weight. To permit uniformity of interpretation, radiocalcium data have been presented as per cent recovery of administered dose per unit weight of dry bone and per unit weight of bone calcium.

Table 3 presents balance data showing

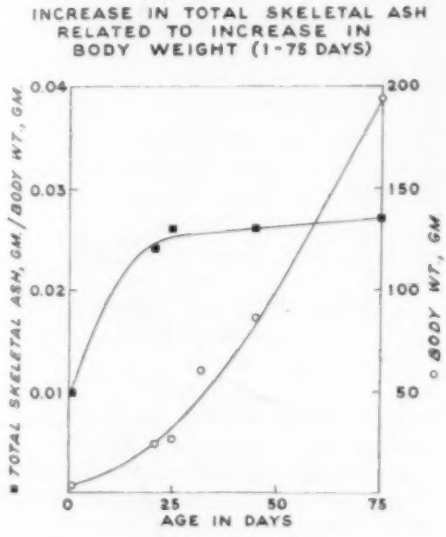
TABLE 3.—*Calcium Balance**

Calcium Source, Mg.	Calcium Mg.	Recovery Per Cen
Skeletal	140	442	74
Dietary	460	0	0
Urine	0	25	4
Fecal	0	131	22
Total	600	308	100

* Data represent the average values per animal (from a total of eight animals).

Comment

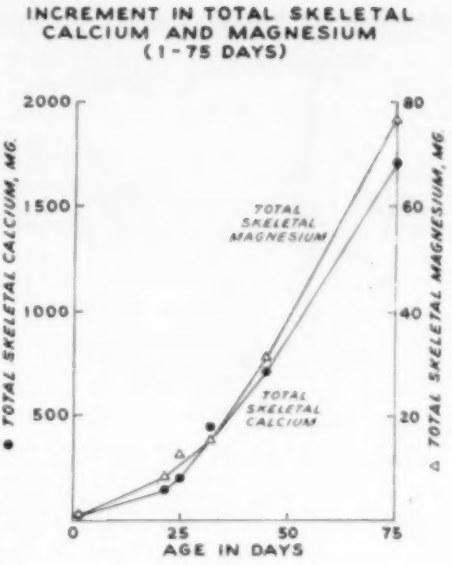
Comparison or rates for general somatic growth (body weight gain) and chemical growth of the skeleton reveals a changing relationship of the two (Table 1). This is perhaps best shown by serial differences in the ratio of body weight to skeletal ash weight throughout the study (Graph 1).



Graph 1.

During the first 21 to 25 days of life, there was a disproportionately rapid increase in the ratio of skeletal ash to body weight. Thus, during this period, growth of the skeleton was more rapid than was growth of other major systems combined. After 25 days of age, although there was a continuing rapid increase in each of these two parameters, the ratio between them tended to be constant. The rapid growth in total mass of skeletal calcium and magnesium from 1 to 75 days is shown in Graph 2.

The total skeletal calcium content also increased in a disproportionately rapid manner with respect to body weight, as would be expected from the data pertaining to skeletal ash. (These data are in direct agreement with those presented by Sherman and McLeod[22]). This tendency was exaggerated, however, because calcium became a progressively greater per cent of ash with age, viz., calcium represented 20%, 27%, 32%, and 33% of ash weight at 1, 21, 45, and 75 days, respectively.



Graph 2.

The extreme proclivity of the immature animal for skeletal incorporation and retention of available calcium # was further demonstrated by Ca^{45} recovery studies. Retention of calcium was demonstrated by the recovery of radiocalcium from the total skeleton at 21, 45, and 75 days of age (Graph 3, upper curve).

Nearly 95% of the radiocalcium administered prior to 21 days of life was recovered at 75 days of age. This marked retention occurred despite a continuous skeletal calcium turnover and dilution by growth. In these studies a high rate of calcium turnover has been shown by changes in Ca^{45} content of long-bone aliquots. These changes are best shown by increase in the calculated recovery of radiocalcium from a hypothetical animal whose skeleton consists of only long bones. These calculations were

References 23-25.

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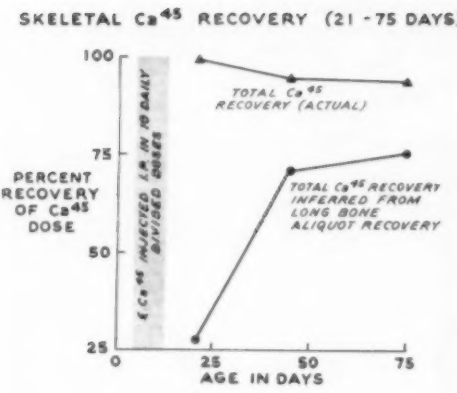
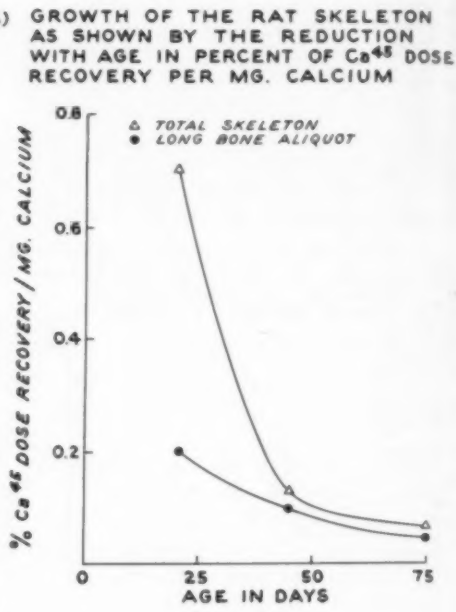


Graph 3.



Graph 4.

projected from the per cent Ca^{45} recovery per unit of calcium in long bones, and the fraction of the total skeletal calcium which the latter represented. As shown in the lower curve of Graph 3, this calculated recovery increased markedly during the period of study. This increase can be explained only in terms of calcium turnover, i.e., exchange of calcium among skeletal parts.

Calcium content of the skeleton during the period of study, in addition to internal exchange, underwent progressive dilution. By 75 days of age, the 12 mg. of calcium contained in the 1-day-old rat was diluted, with negligible loss, by an additional 1700 mg. Based upon the resultant reduction in recovery of Ca^{45} per milligram of total skeletal calcium, a curve depicting this dilution by growth is shown in Graph 4 (upper curve).

During the 21-to-45-day period, the amount of radioactivity detected per milligram of total skeletal calcium underwent a fourfold decrease, in an exactly inverse relation to a proportional increase in total skeletal calcium. (Total skeletal calcium increased from an average of 140 mg. at 21 days to 710 mg. at 45 days.) This relationship continued during the 45-to-75-day period, when the rate of dilution, however, diminished markedly. The nearly 100% retention of Ca^{45} already shown validates this direct relationship between increment in total skeletal calcium and reduction of Ca^{45} per unit of total skeletal calcium.

In contrast to the above data pertaining to the total skeleton, changes in the long bones were less extreme (lower curve, Graph 4). The rate of change was not so great in the long bones and tended to remain constant during the entire period of study, implying a slower and more uniform chemical growth of the long bones than of the remainder of the skeleton during the early days of life, including the first half of the period studied. This is borne out by the fact that uptake of available calcium from the metabolic pool during the period of radiocalcium injection was less per unit of calcium present in long bones than in the skeleton as a whole, as demonstrated by the marked difference between the two curves in Graph 4 at the 21-day points.

Differences and changes in the curves in Graph 3 and Graph 4 correlate well with differences and changes in the calcium content of the ash of the total skeleton compared with that of long-bone aliquots. The calcium content of ash in the long bones was

initially relatively high and remained so (Table 2). The calcium content of the total skeletal ash approached these high levels only during the latter period of the study (Table 1).

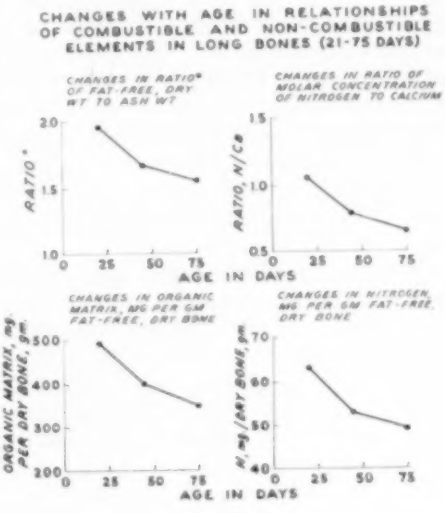
With respect to chemical growth, these data indicate that maturity of bone is related to calcium content. As the calcium content of bone increases, propensity for calcium uptake decreases, that is, the incorporation of additional calcium (chemical growth) is inversely related to the degree of maturity.

A balance study (Table 3) was carried out to determine the quantity of calcium necessarily ingested during this period to implement the rapid chemical growth shown above. During the period 21 to 32 days, the total skeletal calcium of these animals increased by more than 200%; the 300 mg. of calcium so retained represented 65% of calcium ingested. The remaining 35% of ingested calcium appeared in the excreta and was relatively undiluted by skeletal calcium lost through turnover. This is corroborated by Ca^{45} recovery data already presented, which demonstrate a nearly absolute retention of skeletal calcium (Graph 3, upper curve) despite a high rate of turnover during the period studied (Graph 4).

Chemical growth of the total skeleton grossly reflects the combined changes of many centers, each of which at any one time is undergoing growth and changes in chemical composition at an intrinsic rate. There is a tendency toward a uniform chemical composition with increasing age, but at any given time the degree of maturity, and therefore chemical composition, may vary among skeletal parts and within individual skeletal parts. Results of analyses of individual parts during any one period of growth would not therefore reflect the composition of the entire skeleton.*

The present studies have shown that during the early weeks of life, significantly less additional calcium was incorporated per unit of calcium already present in long bones than in the skeleton as a whole. Moreover, these and other discrepancies between long bones and the total skeleton were found to be related to differences in chemical composition.

Detailed analyses of changes in chemical composition of long-bone aliquots have been conducted and data from these studies have been presented in Table 2. Changes with age in the ratio of fat-free dry weight to ash weight of long-bone aliquots revealed a progressive increase in ash per unit weight of bone and a corresponding relative reduction of combustible elements. Organic matrix, which can be grossly estimated by the difference between fat-free dry weight and ash weight of bone,[29] was reduced from 49% of long-bone dry weight at 21 days to 35% at 75 days. The reduction with age in nitrogen per unit of fat-free dry bone paralleled this decrease in combustible matter. These relationships are presented in Graph 5.


CHANGES WITH AGE IN RELATIONSHIPS OF COMBUSTIBLE AND NON-COMBUSTIBLE ELEMENTS IN LONG BONES (21-75 DAYS)
CHANGES IN RATIO* OF FAT-FREE, DRY WT. TO ASH WT.
RATIO*
CHANGES IN RATIO OF MOLAR CONCENTRATION OF NITROGEN TO CALCIUM
RATIO, N/Ca
AGE IN DAYS
CHANGES IN ORGANIC MATRIX, MG. PER GM. FAT-FREE, DRY BONE
ORGANIC MATRIX, mg. PER DRY BONE, gm.
CHANGES IN NITROGEN, MG. PER GM. FAT-FREE, DRY BONE
N, mg./DRY BONE, gm.
AGE IN DAYS

Graph 5.

The apparent decrease in nitrogen (as representative of the organic matrix) during growth was primarily the result of a more rapid increase in noncombustible minerals, chiefly calcium, as shown by a decreasing nitrogen-calcium ratio with age.

* References 3-5, 7, 8, and 26-28.

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Changes in molar concentrations of certain major bone constituents, as related to calcium, are presented in Table 4.

TABLE 4.—*Relationships of Calcium to Other Major Elements in Long Bones, Twenty-One to Seventy-Five Days (Molar Concentrations)*

Age, days	Ca/P	Ca/Mg	Ca/Na	Ca/K	Ca/N
21	1.3	16	22	50	0.96
45	1.5	21	24	135	1.42
75	1.5	23	29	280	1.65

The decrease with age in potassium per unit of bone (Table 2), magnified by a simultaneous increase in calcium, produced a marked progressive increase in the calcium-potassium ratio. This change in potassium concentration paralleled the reduction in organic matrix described above, indicating that potassium was derived chiefly from organic matrix.

The increase in calcium as a per cent of ash related directly to a gradual relative decrease in concentration of phosphorus, magnesium, and sodium. This is reflected by the increase with age of the molar ratio of calcium to each of these elements. The relationship of calcium to phosphorus remained most constant. The molar calcium-phosphorus ratio increased by only 15% from 21 to 45 days, and remained constant thereafter.

Based on the calcium-magnesium ratio in long-bone aliquots and the assumption that at a given age no higher skeletal concentration of magnesium occurs than that in long bones, skeletal magnesium can account for no more than 65% of the total magnesium recovered at 21, 45, and 75 days of age.

Chemical composition of long-bone aliquots tended to become uniform with age, corroborating the earlier work of Hammett.[5] Differences between the chemical composition of long-bone aliquots and the total skeleton, characterizing the early weeks of life, had nearly disappeared by 75 days. However, these discrepancies in chemical composition demonstrate the fallacy in making a projected estimate of total skeletal composition from an analysis of select bones until chemical growth activity and composition have reached uniformity throughout the skeleton.

Summary and Conclusions

The dynamics of chemical growth of the skeleton have been investigated in 95 normal immature male Wistar rats (1 to 75 days of age) by serial analyses of the total skeleton and long-bone aliquots, radiocalcium (Ca^{45}) recoveries, and balance technique.

Chemical growth of the rat skeleton, as represented by increment in total skeletal ash, calcium, and magnesium from birth to 75 days, is disproportionately rapid when compared with other major systems combined. The calcium present at birth (12 mg.) is diluted by an additional 1700 mg. by 75 days of age.

Approximately 65% of ingested calcium ultimately enters the skeletal pool during this period of growth.

As shown by Ca^{45} studies, calcium incorporated into the skeleton is retained nearly 95% by the growing animal, despite a high rate of skeletal turnover.

This early period of life is characterized by continuous changes in the chemical composition of bone. There is an increase in the mineral elements of the skeleton and a relative decrease in organic matrix. In addition, a progressive change in the chemical composition of ash occurs, chiefly represented by an increasing calcium content.

Rates of change in chemical growth and turnover are not equal in long bones and the entire skeleton. Until chemical growth activity has reached uniformity throughout the skeleton, it is fallacious to estimate magnitude or change in the former from analyses of select parts.

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Chemical Growth Dynamics of the Skeleton in the Immature Rat

II. Effects of Vitamin D Deficiency

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and
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Introduction

The present report deals with the effects of vitamin D deficiency on the dynamics of chemical growth and composition during the first 75 days of life in the immature male Wistar rat. In the normal animal this early period of life is characterized by specific changes in chemical composition of bone, and these changes have been correlated with chemical growth and turnover and linear growth in other publications in this series.*

Many studies have been reported which deal with the effects of vitamin D deficiency on somatic and skeletal growth,† skeletal morphology and maturation,‡ and on chemical composition and turnover of bone.§ The following studies were undertaken to correlate chemical growth and turnover of the entire skeleton with serial changes in the chemical composition and linear growth of long bones in animals maintained on vitamin D-deficient and normal diets.

Materials and Methods

Male Wistar rats were used throughout this study. Litters were arranged at birth to contain approximately 8 to 10 male animals. All animals were weaned at 21 days of age to a vitamin D-deficient diet (Diet 2) ‖ which contained 0.76% calcium and 0.42% phosphorus. A constant source of distilled water was supplied. Animals were killed at 21, 45, and 75 days of age for analysis of either total-skeleton or long-bone aliquots.

In certain groups carrier-free radiocalcium (Ca^{45}) was injected daily, intraperitoneally, in 10 divided doses totaling 20 μc, prior to 21 days of age, in order to permit an associated analysis of total and differential skeletal uptake and turnover of calcium.

Methods pertaining to the preparation of the skeleton and skeletal aliquots for analysis, techniques of chemical analysis, and radiocalcium determinations were identical to those already reported.[1]

Results

Data pertaining to the comparison of chemical growth of the total skeleton of the animals maintained on the vitamin D-deficient diet with that of control animals are presented in Table 1.

Data obtained by analyses of long-bone aliquots from other D-deficient groups of

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Presented in part before the Western Society for Pediatric Research at Portland, Ore., October, 1955.

* References 1 and 2.
† References 3-5.
‡ References 6-10.
§ References 11-17.

‖ This diet contained no vitamin D. The composition regarding carbohydrate, protein, calcium, phosphorus, and magnesium was identical with that of the diet used in the study of control animals (Diet 1), described in a previous publication (Reference 1).

TABLE 1.—*Comparison of Total Skeletal Calcium in Animals on Normal and Vitamin D-Deficient Diets at Twenty-One, Forty-Five, and Seventy-Five Days of Age*

	Normal Diet*					
Age (days)	21		45		75	
No. of animals	11		5		5	
	Av.	Range	Av.	Range	Av.	Range
Skeletal calcium						
Total mg.	142	112–167	710	676–742	1710	1530–1890
Mg/gm. body wt.	6.0	4.4–6.9	8.3	7.3–9.3	8.9	8.0–9.1
Ca^{45}, % recovery	99	91–112	94	89–96	93	82–98
	D-Deficient Diet†					
Age (days)	21		45		75	
No. of animals	11		6		6	
	Av.	Range	Av.	Range	Av.	Range
Skeletal calcium						
Total mg.	142	112–167	600	560–660	1510	1180–1750
Mg/gm. body wt.	6.0	4.4–6.9	7.0	6.7–7.2	7.9	6.2–9.5
Ca^{45}, % recovery	99	91–112	77	75–79	65	64–72

* Diet 1, which consisted of a standard normal diet developed by the Institute of Experimental Biology, University of California at Berkeley, Calif., where it has been used extensively in growth studies. Its composition was described in a previous publication (Reference 1).

† Diet 2, which was essentially identical with Diet 1 except for a complete Vitamin D deficiency.

animals at 21, 45, and 75 days of age are presented in Table 2.

Averages presented in Table 1 and Table 2 have, in general, been rounded off to two significant figures. All individual elements have been reported in relation to extracted dry weight and ash weight of bone aliquots, with the exception of nitrogen which is related only to dry weight. To permit uniformity of interpretation, radiocalcium data have been presented as per cent recovery of administered dose per unit

TABLE 2.—*Chemical Composition of Long Bone Aliquots in Animals on Vitamin D-Deficient Diet at Twenty One, Forty-Five, and Seventy-Five Days of Age*

Age (days)	21		45		75	
No. of animals	15		12		8	
	Av.	Range	Av.	Range	Av.	Range
Body wt., gm.	26	17–36	90	67–117	190	122–223
Tibial diaphyseal length, mm.*	15.4	15.0–15.7	25.0	23.7–27.0	31.7	30.0–32.2
Long bone						
Dry wt/ash wt.	1.96	1.94–2.08	1.67	1.64–1.70	1.54	1.53–1.59
Dry wt. − ash wt.	0.49	0.46–0.52	0.40	0.39–0.41	0.35	0.35–0.37
Dry wt.						
Calcium						
Mg/gm. dry †	170	162–177	260	180–220	216	210–220
Mg/gm. ash †	330	317–346	340	300–384	334	325–344
Magnesium						
Mg/gm. dry	5.9	4.2–6.7	6.7	5.6–8.3	5.5	4.0–8.3
Mg/gm. ash	12.5	12.0–13.0	11.1	9.4–14.0	8.6	6.7–13.0
Phosphorus						
Mg/gm. dry	98	97–101	110	100–115	120	114–125
Mg/gm. ash	190	190–197	180	170–190	190	175–193
Sodium						
Mg/gm. dry	4.5	4.4–4.7	5.1	4.2–6.3	4.3	3.7–4.8
Mg/gm. ash	8.8	8.5–9.3	8.6	7.3–10.3	6.6	5.7–7.5
Potassium						
Mg/gm. dry	3.4	2.8–4.1	1.6	1.2–2.2	0.84	0.5–1.1
Mg/gm. ash	6.7	5.5–8.1	2.6	2.1–3.7	1.3	0.8–1.6
Nitrogen						
Mg/gm. dry	63	61–66	49	47–53	47	44–52
Ca^{45} recovery ‡						
%/gm. dry	35	32–35	23	17–26	11	10–13
%/mg. Ca	0.20	0.18–0.21	0.12	0.09–0.14	0.05	0.05–0.06

* Data pertaining to tibial length are presented in detail in a subsequent publication in this series (Reference 2).

† "Dry" and "Ash" refer to the dry weight and ash weight of skeletal aliquots.

‡ Ca^{45} data were obtained from only eight animals at 45 days and five animals at 75 days.

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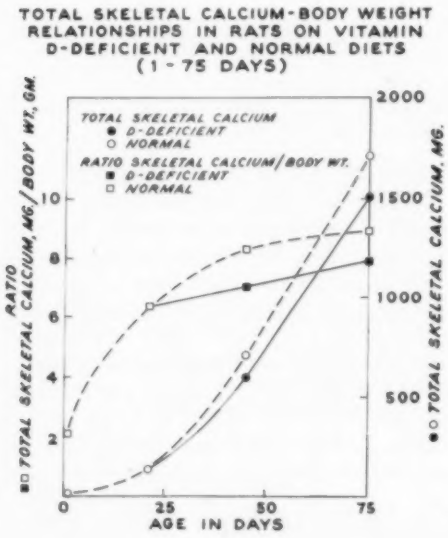
weight of dry bone and per unit weight of bone calcium.

Comment

General somatic growth, as represented by body weight gain, was normal in the D-deficient groups studied. However, increment in total skeletal calcium lagged from normal in these animals. The changing relationships between increment in total skeletal calcium and body weight gain in the animals maintained on the D-deficient diet are compared with those of normals in Graph 1.



Graph 1.

Relative to body weight gain, the D-deficient animals retained less skeletal calcium than the normals. After 24 days on the D-deficient diet, a 16% deficit of total calcium had accrued. This deficit persisted throughout the period of study, and by 75 days of age it averaged 200 mg. of calcium per animal, a difference of suggestive significance (P value, 0.06).

The animals on the vitamin D-deficient diet contained considerably less total magnesium than normal animals at 45 and 75 days of age, as shown in Table 3.

The total magnesium deficit of 28 mg.

TABLE 3.—*Comparison of Magnesium Recoveries in Animals on Control and Vitamin D-Deficient Diets*

Diet	Magnesium	Age (days)			
		1	21	45	75
1*	Total mg.	1	8	31	76
	Mg/gm. body wt.	0.19	0.32	0.36	0.40
2†	Total mg.	1	8	23	48
	Mg/gm. body wt.	0.19	0.32	0.26	0.25

* Diet 1, the standard normal diet.
† Diet 2, the vitamin D-deficient diet.

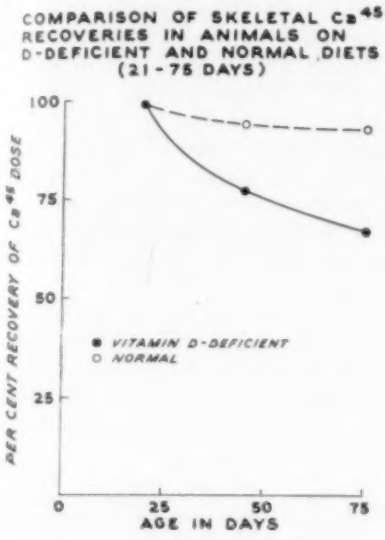
in the 75-day-old animals on the D-deficient diet represents a 40% reduction from normal. The concomitant reduction in magnesium content of long bones (Tables 2 and 3) can account for no more than 10% of the total 28 mg. magnesium deficit shown. As a result, skeletal magnesium in the animals on the D-deficient diet represented a higher per cent (up to 80%) of recovered magnesium than it did in the control animals (in which no more than 65% of the total magnesium was derived from bone). It is evident therefore that a very sizable magnesium deficit developed in skeletal muscle, the only system other than skeleton contributing significantly to the total magnesium of the skinned, bled, and eviscerated preparation used.

Radiocalcium recovery data presented in Table 1 revealed that by 75 days of age the animals on the D-deficient diet incurred a net loss of 33% of the Ca^{45} incorporated into the skeleton prior to 21 days of age, in contrast to a loss of only 7% from normal animals over the same period [1] (Graph 2).

This excessive loss of endogenous calcium in the animals on a D-deficient diet is consistent with other reports, indicating that an exaggerated calcuria occurs in rickets despite reduction in gastrointestinal absorption of calcium.¶

Skeletal calcium turnover has been estimated by changes in the per cent Ca^{45} re-

¶ References 14, 16, 18, and 19.



Graph 2.

covery per unit of calcium in long bones (Table 2) and the fraction of the total skeletal calcium which the latter represented. In the studies of normal animals during the first 75 days of life, the marked retention of Ca^{45} occurred despite a high rate of calcium turnover.# Paradoxically, while the animals which were maintained on the D-deficient diet underwent a 25% greater loss of total radiocalcium (corroborating the observation of Copp and coworkers [16]), the rate of long-bone-calcium turnover in these animals was nearly identical to that of normals.

With increasing age in normal rats the total radiocalcium recovery estimated from Ca^{45} content per unit of long-bone calcium increased markedly, approaching but never equalling the actual skeletal Ca^{45} recovery. In these normal animals, the actual and estimated recoveries at 75 days of age were 93% and 75% of the administered dose, respectively. Conversely, in the animals maintained on the D-deficient diet, the total Ca^{45} recovery estimated on the basis of long-bone analyses equalled the true recovery by 75 days.

These data indicate that the 33% loss of Ca^{45} from the skeletons of the D-deficient animals did not emanate from the long bones. Relative to bones of lower calcium content, the long bones appear less prone to release calcium under the influence of a D-deficient diet.* This fact correlates well with previously published evidence that during the early weeks of life, long bone incorporates relatively less additional calcium from metabolic sources than does the skeleton as a whole.[1] There is an inverse relationship between calcium density of bone and propensity for calcium uptake and release (turnover).† Furthermore, Copp demonstrated a rapid uptake associated with an accelerated release of Ca^{45} by bone in immature rachitic rats,[10] and the present studies show that in growing animals maintained on a vitamin D-deficient diet there is a net deficit in terms of skeletal reincorporation of calcium released from bone to the metabolic pool during turnover. The severity of this endogenous calcium deficit is inversely related to the calcium concentration of bone involved, being most extreme in areas of greatest growth activity.

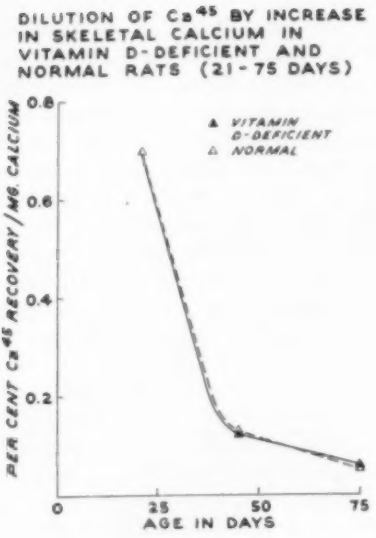
In the normal growing animal, the calcium content of the skeleton underwent progressive dilution from 21 to 75 days of age, with negligible loss of the Ca^{45} tag which was present at 21 days. A comparison of dilution by growth (i.e., by increnormal and the vitamin D-deficient groups ment in total skeletal calcium) in the based upon the reduction in the per cent of Ca^{45} recovered per milligram of total skeletal calcium is shown in Graph 3.

The dilution with age of skeletal Ca^{45} was essentially equal in the normal animals and those maintained on the D-deficient diet. In the light of reduced total skeletal calcium and reduced Ca^{45} recovery in the animals on the D-deficient diet, this sim-

References 1, 20, and 21.

* The calcium density of long bones is appreciably greater than that of the total skeleton prior to 21 days of age. Thereafter, the calcium content of the total skeleton approaches that of long bone (References 1 and 22).

† References 1, 20, 21, 23-26.



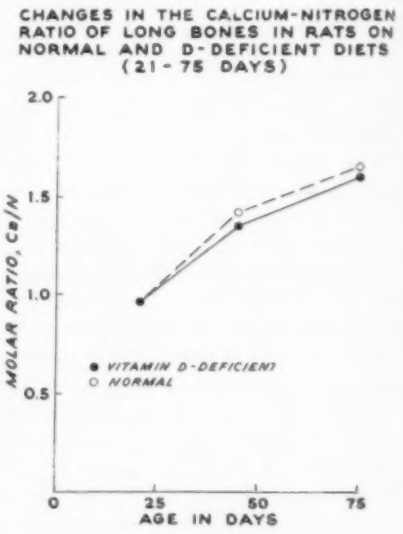
Graph 3.

ilarity of dilution by growth indicates the parallel nature of these losses, and validates the assumption that they are primarily due to loss of calcium from bone with turnover and not to an effect on gastrointestinal absorption. The predominance of the skeletal effect of vitamin D in these studies is in agreement with reports by others who have shown, in experiments minimizing the effects of vitamin D on gastrointestinal absorption, that vitamin D produced increased retention of calcium by bone.‡ The present studies differ from those earlier investigations (which employed low calcium diets) in that they involve no alteration from normal in calcium intake.

Tibial lengths of the animals on the vitamin D-deficient and normal diets were measured and found indistinguishable. Histological studies in the animals on the D-deficient diet revealed only minimally suggestive early rachitic changes in the tibial epiphyses. The details of these studies will be presented in a subsequent publication.[2]

Detailed analyses of changes in chemical composition of long-bone aliquots in the animals on the D-deficient diet have been conducted for correlation with gross skeletal changes and comparison with normals (Table 2). Changes with age in the ratios of fat-free dry weight to ash weight of long-bone aliquots and of organic matrix to fat-free dry weight were identical in the two groups studied. Similarly, the reduction with age in nitrogen per unit of fat-free dry bone was parallel in the two groups.

The increase with age in bone ash, reflected chiefly by calcium, and the related decrease in organic matrix, as represented by nitrogen, was equal in the two groups. These relationships are presented for comparison in Graph 4.



Graph 4.

The similarity in bone composition in the normal and D-deficient groups was also found among mineral elements, with the exception of calcium and magnesium, in which minor differences were noted. By 75 days, calcium and magnesium concentrations in the long bones of the D-deficient groups lagged 6% and 10%, respectively. This amount, in either case, can account for only a small per cent of the over-all deficit of these two elements, and did not

‡ References 14, 17, and 27.

significantly alter the relationship of calcium to other major bone constituents.

The similarity of changes in molar concentration of major bone constituents as related to calcium in the animals on the D-deficient and normal diets are presented in Table 4.

TABLE 4.—*Relationships of Calcium to Other Major Elements in Long Bones of Rats on D-Deficient and Normal Diets, Twenty-One to Seventy-Five Days (Molar Concentration)*

	Vitamin D-Deficient Diet				
Age (days)	Ca/P	Ca/Mg	Ca/Na	Ca/K	Ca/N
21	1.3	16	22	50	0.96
45	1.4	19	23	130	1.35
75	1.4	25	29	275	1.60
	Normal Diet				
Age (days)	Ca/P	Ca/Mg	Ca/Na	Ca/K	Ca/N
21	1.3	16	22	50	0.96
45	1.5	21	24	133	1.42
75	1.5	23	29	280	1.65

The failure of the animals maintained on the D-deficient diet to manifest classical rachitic alterations in chemical composition and morphology of long bones no doubt relates largely to the normal phosphorus content (0.42%) of the diet used. It has been demonstrated that histological and major chemical manifestations of florid rickets due to D-deficiency and low phosphorus intake can be reversed by increasing dietary phosphorus to normal.§

In summary, the animals maintained on the D-deficient diet accrued a sizable skeletal calcium deficit, the magnitude of which was not paralleled by the differences from normal in the chemical composition of the long bones. This failure of the long bones in the vitamin D-deficient groups to reflect the over-all skeletal loss with turnover of calcium and its radiocalcium tag is paradoxical, and further emphasizes the previously described fallacy inherent in making projected estimates of total skeletal composition from analyses of select bones in the growing animal.[1]

§ While the studies referred to demonstrated healing of rickets histologically by addition of phosphorus to otherwise rachitogenic diets, correction thereby of the chemical lesion was incomplete; references 3, 27-33.

Summary and Conclusions

The effects of a vitamin D-deficient diet on the dynamics of chemical growth and composition of the skeleton (from 21 to 75 days of age) have been investigated in immature male Wistar rats by serial analyses of the total skeleton and long-bone aliquots, and radiocalcium (Ca^{45}) recoveries.

In these animals maintained on a D-deficient diet, a sizable magnesium deficit developed, with only minor losses from bone, implying that the deficit occurred in skeletal muscle.

A 16% calcium deficit developed in these D-deficient animals by 75 days.

As shown by radiocalcium studies at 21, 45, and 75 days of age, a significantly greater loss of total skeletal Ca^{45} occurred in these animals than in controls.

The parallel nature of losses of skeletal calcium and its Ca^{45} tag indicates that they originated primarily from bone during turnover.

Chemical composition of long bone did not vary significantly from normal and therefore did not reflect the over-all skeletal losses.

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Chemical Growth Dynamics of the Skeleton in the Immature Rat

III. Correlation of Growth and Morphology of Long Bones with Chemical Growth in Normal and Vitamin D-Deficient Animals

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Introduction

This publication presents data pertaining to linear bone growth and morphology in immature male Wistar rats on normal and vitamin D-deficient diets and correlates these data with chemical growth and turnover of the skeleton, previously reported.* Studies involving linear growth, histology, and chemistry of long bones have long been accepted individually as representative indices of the influences of metabolic and endocrine factors upon the skeleton.† However, there has been a paucity of studies correlating these parameters, particularly as they relate to total skeletal chemistry and turnover. The present series of studies were undertaken to provide a correlated chemical, radiological, and morphological interpretation of skeletal growth from birth through 75 days of age in male Wistar rats maintained on normal and vitamin D-deficient diets. Furthermore, these studies bring to focus the observations of earlier workers suggesting that the chemical changes induced in bone by a vitamin D-deficient diet may be dissociated from the effects upon linear growth and morphological variation.‡

Materials and Methods

The general procedure in this series of investigations has involved study of male Wistar rats killed at 1, 21, 45, and 75 days of age. Two major groups have been investigated, consisting of animals maintained on either a normal or a vitamin D-deficient diet.§ Groups of animals at each age were evaluated with respect to either total skeletal chemical growth and turnover or long-bone chemical composition and turnover and histological and roentgenological features.

Specific materials and methods used in these studies, and resultant data pertaining to chemical composition, growth and turnover in animals maintained on the normal and D-deficient diets have been reported in detail.‖ Representative values from these published data are reduplicated in the present report for further correlation.

For x-ray and histological studies, extremities were placed in 10% formaldehyde solution (Formalin) at autopsy. Tibiae were later dissected free of soft tissues and x-rays taken in a uniform manner with Kodak Super Speed Occlusal Dental Film and the following technique: 30 K.V., 50 ma., no filter, .08-in. TFD, and one-secoad exposure time. Film was developed for five minutes with x-ray film developing solution. Diaphyseal lengths were measured directly from the films. Preparation for

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† References 3-7.

‡ References 8-14.

§ The normal (Diet 1) and D-deficient (Diet 2) diets were identical in composition regarding carbohydrate, protein, calcium (0.76%), phosphorus (0.4%), and magnesium. They differed only by absence of vitamin D in Diet 2.

‖ References 1 and 2.

histological study involved decalcification in 5% nitric acid in formaldehyde solution for three to five days, washing, imbedding in paraffin, sectioning at 6μ, and staining with hematoxylin and eosin.

Results

The radiological appearance of tibiae was remarkably uniform among normal animals in each age group. Moreover, despite marked variation in chemical composition and turnover, there were no discernible differences at 45 and 75 days between x-rays of animals on the normal and the D-deficient diets. A representative x-ray of each group at 75 days of age is shown for comparison in Figure 1.

Fig 1.—*A*, x-ray of normal rat tibia at 75 days of age. *B*, x-ray of vitamin D-deficient rat tibia at 75 days of age. Note the absence of discernible differences between these x-rays.

In addition to similarities in radiological configuration among all animals from 1 to 75 days, tibial diaphyseal lengths increased uniformly and no significant differences were observed between groups maintained on normal and vitamin D-deficient diets (Table).

Tibial Diaphyseal Length in Animals on Normal and Vitamin D-Deficient Diets (One to Seventy-Five Days)

	Normal Diet			
Age (days)	1	21	45	75
No. of animals	5	6	10	9
Diaphyseal length, mm. Mean	3.9	15.4	24.7	31.1
σ	0.2	0.2	0.4	0.7
	D-Deficient Diet			
Age (days)	1	21	45	75
No. of animals	5	6	10	9
Diaphyseal length, mm. Mean	3.9	15.4	25.0	31.7
σ	0.2	0.2	1.2	0.7

Grossly and microscopically there were no discernible differences between the tibiae of the normal and vitamin D-deficient animals at 45 days (after 24 days on Diet 2). At 75 days, although grossly similar, certain minimal yet significant microscopic changes were noted in the bones of the deficient animals. These consisted of a slight increase in the over-all thickness and cellularity of the epiphyseal cartilage, associated with an irregularity in alignment of the hypertrophic cartilage cells. No significant microscopic alteration was noted in the formation of the primary bone trabeculae or in bone resorption and remodeling with respect to bone cellularity and matrix (undecalcified sections were not studied). These features are demonstrated in Figure 2 which presents photomicrographs of tibiae of normal and D-deficient animals at 75 days of age.

Comment

Chemical growth of the skeleton in the normal immature male Wistar rat, as represented by increment in total calcium, is extremely rapid in the first 75 days of life, during which time total calcium increases from 12 to 1700 mg. There is a rapid increase in the ratio of skeletal mass to body weight during the first three to four weeks of life; thereafter, a more constant relation-

Fig. 2A.—Epiphyseal cartilage and diaphysis of the tibia in a 75-day-old normal Wistar rat.

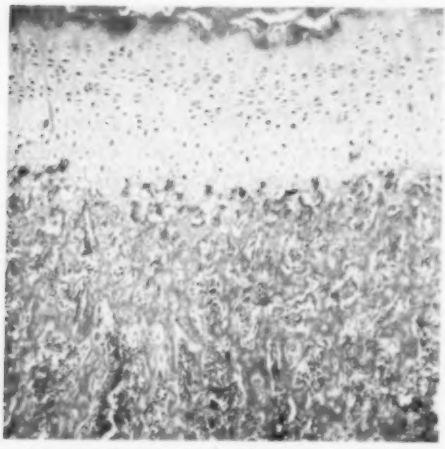
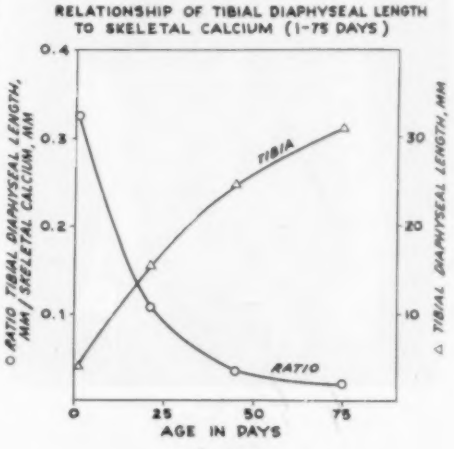


Fig. 2B.—Epiphyseal cartilage and diaphysis of the tibia in a 75-day-old vitamin D-deficient Wistar rat. There is a slight increase in the thickness and cellularity of the epiphyseal cartilagenous plate while the columns of hypertrophic cartilagenous cells are somewhat irregular in alignment as compared with the normal (Fig. 2A).

ship is approached. Because of this changing relationship, estimations of skeletal mass based on body weight are not considered reliable in the immature animal. Moreover, to attempt predictions of the total calcium or other individual elements in skeletal mass from body weight is even less reliable, because progressive changes occur with age in the chemical composition of the skeleton.

In addition to the above changes with respect to the total skeleton, analysis of long bones also reveals a changing chemical composition; at any age from 1 to 75 days, the composition of long bones is not representative of the skeleton as a whole.[1] These features of chemical growth and composition of the rat skeleton and its long bones have been validated by studies involving recovery of a Ca^{45} skeletal tag imposed before 21 days of age, by subsequent reduction in Ca^{45} concentration by increment in total skeletal calcium, and by determination of radiocalcium turnover in long bones.[1]

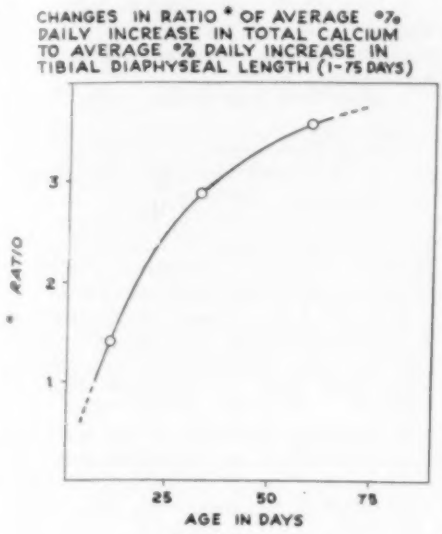
An analysis of the relationships between tibial diaphyseal length and features of skeletal chemical growth and composition is particularly germane to investigations dealing with bone because of the common practice of accepting changes in tibial length in lieu of chemical analyses.[5] No consistent relationship between tibial diaphyseal length and total skeletal calcium occurred in animals included in the present study from 1 to 75 days of age, as shown in Graph 1.



Graph 1.

Furthermore, comparison of the rates of change of these two indices (total skeletal calcium and tibial length) of skeletal growth revealed a disproportionately rapid rate of increase of skeletal calcium. This is shown by changes in the ratio of the average daily per cent increase in total skeletal

calcium to the average daily per cent increase in tibial length (Graph 2).



Graph 2.

Because of the inconstancy in the rate of increase in skeletal calcium as compared with tibial length, interpretation of skeletal chemical growth by inference from change in linear proportions (tibial length, tail length, etc.) would not be valid. Chemical growth of the total skeleton grossly reflects the combined changes of many centers, each of which at any one time is undergoing growth and changes in chemical composition at an intrinsic rate. The composition of the total skeleton, at any one time, is not reflected in long-bone composition, and certainly not in long-bone length. In the animals maintained on the vitamin D-deficient diet, a significant deficit in total skeletal calcium accrued by 75 days.[2] As shown in the Table, this was not attended by any variance from normal in tibial diaphyseal length, further emphasizing the fallacy inherent in estimating chemical growth from linear measurements.

Despite significant changes in chemical growth and turnover in the D-deficient animals, no differences from normal were seen radiologically, and no more than minimal evidence of rachitic processes were seen histologically (suggestive "overgrowth" of epiphyseal cartilage). The absence of the characteristic morphological, roentgenological, and linear growth features of florid rickets apparently resulted from the adequate phosphorus content of the D-deficient diet employed in these investigations.¶

In summary, these data show that the effects of vitamin D-deficiency on chemical growth and turnover of the skeleton can be of significant proportions in the absence of significant alterations from normal in linear growth, chemical composition, roentgenological or histological appearance of long bones.

Summary and Conclusions

Tibial growth and morphology have been evaluated in immature male Wistar rats on normal and vitamin D-deficient diets and have been correlated with skeletal chemical growth and turnover.

The per cent daily increment in tibial diaphyseal length was not proportional to the per cent increase in skeletal calcium from 1 to 75 days of age.

While maintenance of these immature animals on a D-deficient diet induced significant chemical changes, these changes were in no way reflected by alterations in linear growth of tibiae, and were accompanied by only minor histological changes.

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Case Reports

CUSHING'S SYNDROME IN CHILDREN ASSOCIATED WITH ADRENAL CORTICAL CARCINOMA

A Case Report with Review of the Literature

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THE ASSOCIATION of adrenal tumors with the clinical picture now identified as Cushing's syndrome was first observed by Cooke * in 1756. Cushing,[2] in 1932, collected 12 similar cases and concluded that pituitary basophilism was the underlying lesion. Cushing's original concept has been modified as a result of subsequent investigations.† In the pediatric age group Cushing's syndrome is most frequently caused by malignant adrenal tumors. Basophilic adenomas of the pituitary, while rare, have been reported in children in the absence of adrenal pathology.‡ The only case of Cushing's syndrome in a child caused by adrenal hyperplasia was described by Chute and his colleagues [14] and occurred in a girl 8 years of age. Albright and Forbes § believe that all cases of Cushing's syndrome in infancy are caused by malignant adrenal tumors. Recently, however, Powell [15] has observed an infant, age 3 months, with Cushing's syndrome who was found at autopsy to have bilateral adrenal adenomas.

It is the purpose of this paper to review the reported cases of carcinoma of the adrenal cortex causing Cushing's syndrome in children and to add a case studied in this hospital which is believed to be the youngest case reported to date.

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* Cooke, W., cited by Hicks, J. B.[1]

† References 3-8.

‡ References 5, 9-13.

§ Albright, F., and Forbes, A. P., personal communication with the authors.

Report of Case

A 3-month-old white girl was admitted to The Children's Hospital of the District of Columbia on Aug. 31, 1954, because of obesity beginning 6 weeks prior to admission. The family history revealed that the mother, age 27 years, had suffered from diabetes since the age of 4 years. During her adolescence the disease was controlled with difficulty, and she experienced many episodes of diabetic coma. Following coma, her first pregnancy terminated in May, 1951, by the delivery of a 1136 gm. (2½ lb.) stillborn boy. The patient herein described was the result of the second gestation. There were no episodes of coma during the course of this second pregnancy. The mother's blood sugar during this pregnancy ranged from 79 to 165 mg/100 ml., dropping to 54 mg/100 ml. on the day of delivery. During the day of delivery the total 24-hour excretion of glucose was 150 mg. The maximum daily dose of insulin was 105 units. No additional hormone therapy was given during pregnancy. The mother at 8 months' gestation was delivered by Cesarean section of a girl weighing 2700 gm. (6 lb. 3 oz.). The infant's blood sugar was low at birth but rose to 120 mg/100 ml. by the third day. For two months prior to admission the infant had been irritable and was treated with phenobarbital. Six weeks prior to admission the parents considered the infant to be obese. For three weeks prior to admission the patient was noted to have an eczematoid and acneform rash on the cheeks. She had been on a low fat formula. Food intake had not been excessive. At 10 weeks she weighed 4100 gm. (9 lb.) and on admission 5500 gm. (12 lb.).

On physical examination the temperature was 99.8 F (rectal), pulse rate 108, and respiration rate 28 per minute. The systolic blood pressure ranged from 115 to 210 mm. Hg. The infant was obese, showing a buffalo hump and moon face. There was no axillary or pubic hair and no abnormality of the external genitalia other than slight clitoral hypertrophy. There was hirsutism of the upper lip and an erythematous papular rash over the face and neck. The heart was not enlarged to percussion, and no murmurs were heard. The abdomen was distended, and the liver was palpable 4.0 cm. below the right costal mar-

gin. A firm mass 4.0 cm. in diameter unattached to the liver was palpated to the right of the umbilicus. X-ray examination revealed marked osteoporosis of the entire skeletal system (Fig. 1). X-rays of the chest and abdomen were normal. An intravenous pyelogram indicated secretory activity of both kidneys. An electrocardiogram was interpreted as showing left axis deviation with prolongation of the Q-T interval. This was thought to represent hypopotassemia.

Fig. 1.—X-ray showing osteoporosis of vertebrae, pelvis, and femora.

The hemogram was as follows: hemoglobin 11.8 gm/100 ml., white blood cells 22,700/cu. mm.; differential count, 67% polymorphonuclear leucocytes, 29% lymphocytes, and 4% monocytes. A total eosinophile cell count was zero per cubic millimeter. The fasting blood sugar was 115 mg/100 ml.; plasma sodium 116.9 mEq/L; potassium 3.0 mEq/L; chlorides 76.0 mEq/L; calcium 10.5 mg/100 ml., blood urea nitrogen 26 mg/100 ml., and CO_2 combining power 22 mEq/L. The urinary excretion of 11-oxycorticosteroids was 2.4 mg. in 12 hours and the 17-ketosteroids 1.7 mg. in 12 hours.‖ For two days prior to surgery and on the morning of the operation she received 50 mg. of cortisone intramuscularly and 30 mEq. of potassium ion by mouth. On the ninth hospital day, while the patient was under hypothermic anesthesia, an exploratory laparotomy was performed. A tumor replacing the right adrenal gland was found and removed. For three days postoperatively the patient's systolic blood pressure ranged between 190 and 210 mm.Hg. Her blood sugar dropped to 55 mg/100 ml. about seven hours postoperatively. She received 10 ml. of adrenal cortical extract, 50 mg. cortisone, intravenous fluids, a blood transfusion, and tetracycline (Achromycin) hydrochloride 75 mg. every eight hours and procaine penicillin 1,200,000 units daily. Approximately four hours following surgery, blebs and bullae appeared on the skin of all extremities. Pseudomonas aeruginosa and Proteus vulgaris were cultured from the surgical wound and from the skin lesions.

Her condition deteriorated on the third postoperative day and clinical signs of pneumonia became evident. There was abdominal distention and a weak cry. The infant died on the fourth postoperative day after several episodes of apnea.

Pathological Summary.—The surgical specimen consisted of a tumor which was encapsulated, ovoid, nodular, moderately soft, pinkish gray, weighing 105 gm. and measuring 8.0×5.5×3.5 cm. On section the tissue was reddish tan and lobulated with softened hemorrhagic areas (Fig. 2).

Microscopically, the cells were polyhedral, forming solid alveolar groups and cords separated by capillaries and thin fibrous tissue septa. The capsule was invaded and areas of hemorrhage and necrosis were present. The majority of tumor cells were of uniform size with large hyperchromatic nuclei and a fair amount of pale cytoplasm. Mitotic figures were rare. No chromaffin was seen following fixation in chrome salts or using Gömöri's stain. Oil Red O stain revealed minute amounts of fat. The diagnosis was cortical carcinoma of the right adrenal gland (Figure 3). The tissue was submitted to Dr. Fred W. Stewart, Memorial Center, New York, who concurred in the diagnosis.

Postmortem examination was performed three hours after death. At autopsy the infant weighed 5557 gm., measured 22 cm., and presented the classical habitus and facies of Cushing's syndrome. A few acneform lesions were noted on each cheek and a thin line of fine dark hair outlined the upper lip. Except for slight clitoral en-

‖ The normal 24-hour excretion of 11-oxycorticosteroids using the formaldehydrogenic method is 0.5-1.5 mg. The normal 24-hour excretion of 17-ketosteroids for this age is less than 1 mg.

Fig. 2.—Right adrenal cortical tumor.

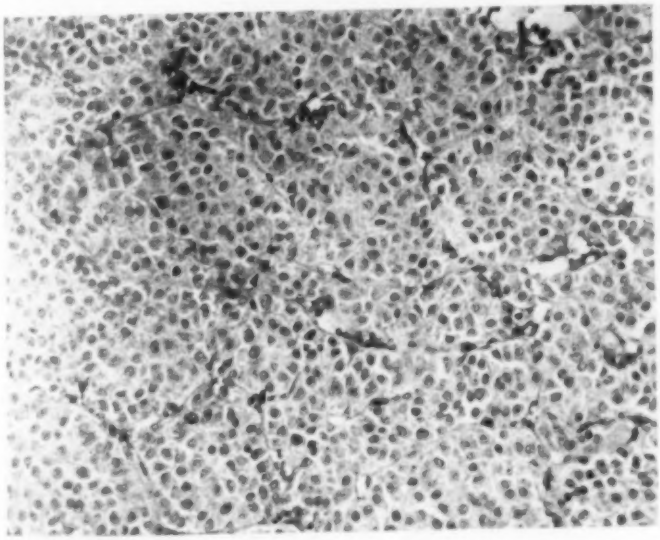

Fig. 3. — Microscopic appearance of adrenal cortical carcinoma; mag. × 115.

largement the genitalia were those of a female infant. No axillary or pubic hair was present. Superficial necrotic areas and blebs measuring up to 1.5 cm. were present over the left heel, right leg, and upper abdomen. Postmortem blood culture grew Pseudomonas aerugenosa. Subcutaneous fat averaged 3.0 cm. over the thorax and 2.5 cm. over the abdomen. The heart was twice the normal weight, and the left ventricle was hypertrophied. Miliary abscesses were present in the lungs and many branches of the pulmonary artery were filled with emboli of tumor cells which were identical in appearance to those seen in the original tumor (Fig. 4). The liver

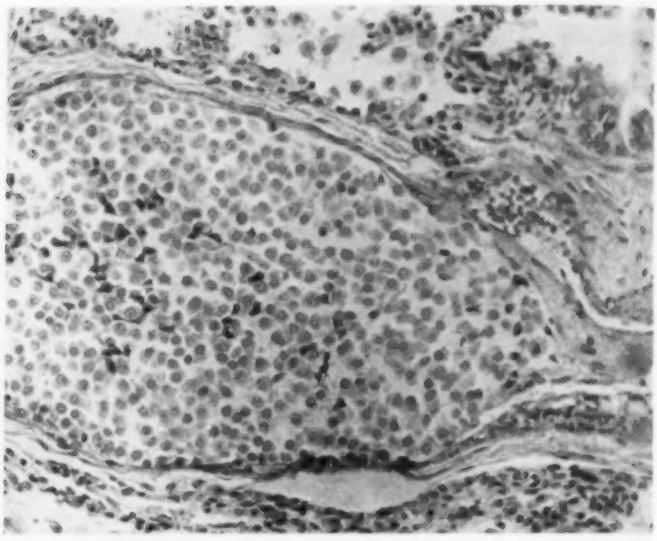

Fig. 4.—Adrenal cortical carcinoma metastatic in blood vessel of lung; mag. × 115.

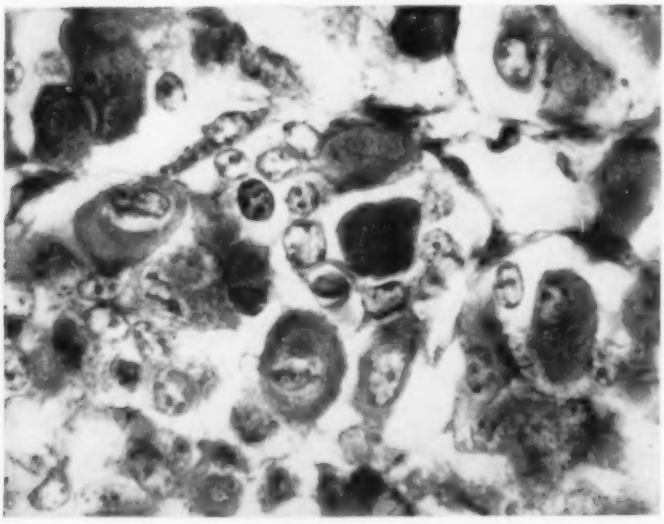

Fig. 5.—Anterior pituitary gland showing Crooke's hyaline change of the basophilic cells; mag. × 650.

was studded throughout with unencapsulated foci of tumor cells similar in appearance to those described above.

The thyroid, thymus, spleen, and lymph nodes were not remarkable. The pancreas weighed 10 gm. and measured 8.5×1.0×0.5 cm. The islets of Langerhans were numerous throughout the gland. The right adrenal was surgically absent. The left adrenal weighed 1.5 gm. and measured 1.0×0.4×0.3 cm. Oil Red O stain revealed more lipid in the zona glomerulosa than in the remaining zones. The kidneys were normal except for a few hyaline casts in the collecting tubules. The vagina, uterus, tubes, and ovaries were infantile. The brain was not remarkable except for a 4.0×3.0×2.0 cm. subdural blood clot overlying the right frontal area. The pituitary gland was grossly normal. Microscopically, the acid-

	Yr. of Publication	Authors	Sex	Age of Patient, Yr.	Age at Onset, Yr.	Typical Obesity	Striae	Hirsutism	Virilism	Blood Pressure	Osteoporosis	Weakness	Increased Blood Sugar	Eosinopenia
	1925	Bullock and Sequeira[17]	F	11	10	+	+	+	+	+	--	--	--	--
	1927	Kennedy and Lister[18]	F	13	12½	+	+	+	--	--	--	--	+	--
	1930	Schweizer and Senet[19]	F	7	6½	+	--	+	+	150/90	--	--	--	--
	1933	Van der Bogert[20]	F	2	9 mo.	+	--	+	+	160/110	--	--	--	--
	1936	Little[21]	F	7½	3½	+	--	+	+	120/80	+	+	--	--
	1936	Rasmussen[22]	M	13	?	+	+	+	+	+	+	--	+	--
	1939	Crooke and Callow[23] (Case 2)	F	6	6	+	+	+	+	180/145	−	−	−	--
	1940	Gross[24] (Case 4)	F	10¼	10	−	--	+	−	160/120	--	+	+	--
	1940	Gross[24] (Case 5)	F	3¼	3	+	--	--	−	260/190	--	--	+	--
	1943	Farber and others[25]	M	15	14	+	+	+	+	190/156	+	+	+	--
	1948	Glanzmann[26]	F	3¾	2	+	+	+	+	175/88	+	--	+	--
	1949	Walters and Sprague[27]	M	1¼	?	+	--	--	+	--	--	--	--	--
	1949	Walters and Sprague[27]	F	3 4/12	?	+	--	--	+	--	--	--	--	--
	1951	Walters and Sprague[28]	F	15 mo.	--	--	--	--	--	--	--	--	--	--
	1951	Walters and Sprague[28]	F	17 mo.	--	--	--	--	--	--	--	--	--	--
	1950	Wilkins[29]	F	5 mo.	3 mo.	+	--	+	+	180/120 210/110	--	--	+	--
	1951	Garrett[30] (Case 1)	F	2	1¼	+	--	+	−	200/160	--	--	+	--
	1951	Garrett[30] (Case 2)	M	2	1	+	−	+	+	--	Delayed ossification	--	--	−
	1951	Garrett[30] (Case 4)	M	12	11½	+	+	+	+	160/110	−	+	+	+
	1952	Talbot[31]	M	8½ mo.	6½ mo.	+	--	+	+	130/90	--	--	−	+
	1952	Talbot[31]	M	6	6	+	--	+	+	130/100	--	--	−	−
	1952	Harrison[32]	F	10	8½	+	+	+	+	150/80 210/150	--	--	−	--
	1952	Picard and others[33]	M	14	13½	+	−	Female distribution	Feminization	185/135 210/140	+	+	+	--
	1952	Wilkins[34]	F	3	2½	+	--	+	+	135/110	--	--	+	--
	1953	Davies[35]	M	2½	2	+	−	+	−	200/140	−	--	−	+
	1953	Grob and others[36]	F	4	1	+	+	+	−	145/110	--	+	−	1%
	1953	Falk[37]	F	10 mo.	10 mo.	+	+	+	−	220/140	+	--	+	--
	1955	Fèvre and others[38]	F	2½	13 mo.	+	+	+	+	120/80	−	--	+	--
	1956	Guin and Gilbert	F	3 mo.	6 wk.	+	+	+	+	190/210 systolic	+	+	+	+

Urinary Secretion of Hormones		External Genitalia	Right Adrenal	Left Adrenal	Crook's Hyaline Change in Pituitary	Pancreas	Histopathology	Status
17-KS	11-OCS							
--	--	Clitoris enlarged	Enlarged	Tumor	--	--	Adrenal cortical carcinoma	Autopsy: metastases to liver and lungs
--	--	Clitoris not enlarged; labia enlarged	Small	Tumor	--	--	Hypernephroma, adrenal cortex	Autopsy: metastases to lungs
--	--	Clitoris and labia enlarged	Tumor	--	--	--	----	Autopsy: invasion of right kidney, metastases to lungs
--	--	Clitoris enlarged; vulva prominent	Tumor	Large	--	--	Adrenal cortical carcinoma	Autopsy
--	--	----	--	Tumor	--	--	Carcinoma of left adrenal	Autopsy: metastases to liver and lungs
--	--	----	--	Tumor	+	--	Carcinoma of left adrenal	Autopsy
Up to 850 mg.	--	Enlarged	--	Tumor	+	--	Carcinoma of left adrenal	Autopsy: metastases to liver, lymph nodes and invasion of left kidney
--	--	----	Tumor	--	--	--	Carcinoma of adrenal cortex	Autopsy
--	--	Normal	--	Tumor	--	--	Carcinoma of adrenal cortex	Autopsy
--	--	+	Tumor	--	+	--	Carcinoma of adrenal cortex	Autopsy
+	--	Enlargement of of penis	--	Tumor	--	--	Histologic description consistent with carcinoma of adrenal cortex	Autopsy
4.1	--	----	--	--	--	--	Carcinoma of adrenal cortex	Surgery
6.2	--	----	--	--	--	--	Carcinoma of adrenal cortex	Surgery
3.0	--	----	--	--	--	--	Carcinoma of adrenal cortex	----
1.1	--	----	--	--	--	--	Adrenal cortical carcinoma	----
6.8—62	--	----	Tumor ? rt. or lt.	--	--	--	Adrenal cortical carcinoma	Surgery and autopsy; metastases to liver and lung
Less than 1 mg.	--	----	Tumor	Normal	--	--	Carcinoma of adrenal cortex	Surgery
--	--	Normal	Tumor	Tumor	−	--	Carcinoma of adrenal cortex	Autopsy: metastases to para-aortic nodes
51.3 60.5	3.5; estrogens greater than 20 rat units	Adult	Tumor	--	--	--	Carcinoma of adrenal cortex	Surgical exploration; metastases to liver; died; No autopsy.
15	--	Enlargement of penis and scrotum	Tumor	--	--	--	Carcinoma of adrenal cortex	Surgical exploration
6.6	0.2	Enlargement of penis	Tumor	--	--	--	Carcinoma of adrenal cortex	Surgical exploration
150-256	--	----	Tumor	Normal	+	--	Carcinoma of adrenal cortex	Autopsy at age 30; metastases to lungs
32	Folliculin 275 γ	Penis small and atrophic; small testis; gynecomastia	--	Tumor	--	--	Carcinoma of adrenal cortex	Surgery, punch biopsy, X-ray; pulmonary metastases
11 2	--	Enlarged penis	Not present	Normal	--	--	Carcinoma of aberrant adrenal tissue in right lobe of liver	Surgical exploration
1.43-8.4	--	Normal	Extreme autolysis	--	−	--	Adrenal cortical carcinoma of aberrant adrenal tissue	Autopsy: umbilical nodule and peritoneal deposits of adrenal carcinoma; metastases to liver
1.4-2.5	--	Normal	--	Tumor	--	--	Adrenal cortical carcinoma	Surgical removal of tumor
10-20	--	----	Tumor	--	--	--	Adrenal cortical carcinoma	Autopsy: No metastases
--	0.55	Enlarged clitoris	Tumor	Not found	+	--	Adrenal cortical carcinoma	Autopsy: No metastases
3.4	4.8	Slightly enlarged clitoris	Tumor	Normal	+	Increased islets	Adrenal cortical carcinoma	Autopsy: Metastases to liver and lungs

ophilic cells were increased in number. The basophilic cells were numerically normal but were increased in size. A · Russell [16] stain revealed hyalinized cytoplasm with enlarged eccentric nuclei (Fig. 5). The pars intermedius and pars posterior were not remarkable.

Comment

Cushing's syndrome, whether in children or adults, reflects a hyperadrenocorticism with respect to cortocoids.[5] The somatic alterations include buffalo type of obesity, plethora, moon face, striae, hirsutism, hypertension, cessation of growth, and atrophy of skin, muscle, and bone. Osteoporosis is commonly found and usually involves the vertebrae, ribs, pelvis, and less frequently the skull. In addition, a diabetic glucose tolerance curve, eosinopenia, and elevated blood and urinary corticoids are found. The fasting blood sugar is usually within normal limits. When diabetes is present it is insulin resistant. Alterations in the hemogram include hemoglobin values between 15 and 17 gm/100 ml., and 5,000,000 to 7,000,000 erythrocytes per cubic millimeter. Total eosinophile counts are usually below 100 per cubic millimeter.[3]

Hyperadrenocorticism is reflected in the increased production of corticosteroids, which causes disturbances in carbohydrate metabolism, and excess of androgenic steroids which results in hirsutism, acne, enlargement of the external genitalia, and other symptoms of virilism. Variation in amounts of these two steroids accounts for the frequent occurrence of mixed forms. It is not our purpose to review the physiopathology of Cushing's syndrome, since it has been amply recorded elsewhere.¶

The literature was searched for all authenticated cases of adrenal cortical carcinoma associated with Cushing's syndrome in children in whom the disease began before the age of 14 years. Twenty-eight cases were found, and the authors have added one of their own, believed to be the youngest reported to date. The data on these patients are presented in the Table. Any attempt to tabulate the cases of adrenal cortical carcinoma with Cushing's syndrome in a comprehensive fashion is limited by the degree of completeness of the reported cases. The cases of Walters and Sprague,[27] although not fully described in the literature, have been cited by Forbes and Albright [28] as authentic cases of Cushing's syndrome due to adrenal cortical carcinoma and have therefore been included.

Several cases of adrenal cortical tumors were omitted from this Table because sufficient clinical or histological evidence was lacking on which to establish an unequivocal diagnosis. Although the cases of Telesius,# Ogle [40] and Pitman,[41] Dickinson,* Weisse,[42] Allibone [43] (two cases) had adrenal cortical carcinoma, they were reported as showing only obesity and hirsutism. These cases have been excluded. The cases of Fox [44] and Birrell [45] have been omitted as they showed only obesity, hirsutism, and clitoral enlargement, while the cases of Cooke,† Bevern and Römkild,‡ Walker,§ and Lundh [48] have been excluded because of insufficient histological description. Kis-Varday [49] recorded a case of neuroblastoma of the adrenal gland in a 17-year-old girl with Cushing's syndrome whose symptoms began at the age of 14. This case was omitted because of the histologic diagnosis.

Of the 29 tabulated cases, 20 were females and 9 were males. Eighteen patients died, and all but one of these came to autopsy; however, histologic confirmation of adrenal cortical carcinoma on this patient had been previously obtained surgically. Nine patients underwent surgery. As of February, 1956, the patient reported by

¶ References 4, 5, 6, and 8.

Telesius, cited by Linser.[50]

* Dickinson, W. H., cited by Bullock and Sequeira.[17]

† Cooke, E., cited by Bullock and Sequeira.[17]

‡ Bevern and Römkild, cited by Neurath.[46]

§ Walker, cited by Richards.[47]

Wilkins [34] is well, without evidence of disease.‖

The range of urinary excretion of 17-ketosteroids is wide in all age groups and may be influenced by a variety of clinical settings.[8] The urinary excretion of 17-ketosteroids was ascertained in 17 patients and found to be elevated in 15. Estimations of plasma and urinary 17-hydroxycorticoids and urinary 17-ketosteroids provides the most accurate measurement of adrenocortical activity.¶ Recently, several investigators # have demonstrated a persistent elevation of the urinary and plasma 17-hydroxycorticosteroids and the urinary 17-ketosteroids after ACTH stimulation in patients with adrenal cortical carcinoma. This is in contradistinction to patients with adrenal cortical hyperplasia and adrenal cortical adenomas and appears to provide an accurate method of differentiating benign from malignant lesions. Forbes and Albright [28] in a study of 90 patients found the highest excretion of 17-ketosteroids in patients with malignant tumors. The incidence of virilism was also high in these patients. A diagnosis of carcinoma of the adrenal cortex is strongly suggested when the 17-ketosteroid excretion exceeds 30 mg. per 24 hours.[58] The alpha 17-ketosteroids are elaborated by both the adrenal cortex and male gonads, whereas the beta 17-ketosteroids are produced only by the adrenal cortex. Talbot [59] and co-workers have suggested that an increase in the excretion of beta ketosteroids occurs in adrenal cortical carcinoma. The 17-hydroxycorticoid excretion was determined in only 4 of the 29 patients and was found to be elevated in each instance.

Only one example [33] of feminization was found in this series of collected cases, and this was presumably the result of increased secretion of estrogens. One other case (Garrett,[30] Case 4) showed increased urinary excretion of estrogens without clinical evidence of feminization.

Crooke [60] noted the association of a hyaline change in the basophilic cells of the pituitary in patients with Cushing's syndrome. This change, however, is not yet fully understood. In the cases tabulated, the hyaline change was noted in six of the eight cases in which it was sought. Eisenhardt and Thompson [61] studied the pituitary glands of 90 patients with Cushing's syndrome and found Crooke's hyaline change to be present in all cases. Hyperplasia of the islet cells of the pancreas has been noted previously in patients with adrenal cortical carcinomas. Heinbecker [62] believed that hyperplasia of the islets of Langerhans in Cushing's syndrome was the result of an imbalance among the acidophilic cells, which he considered to be hypofunctioning. In the present case the picture is further complicated by the fact that the patient's mother was diabetic. Maternal diabetes with the development of carcinoma and Cushing's syndrome in the infant suggests an endocrinological relationship. Hyperplasia of the islets of Langerhans in infants born to diabetic and prediabetic mothers has been extensively studied,* and an increase in the size and weight of the adrenal glands has been observed in these infants.† An increase in the number of eosinophilic cells in the anterior hypophysis ‡ together with hyperplasia of the female sex organs has also been described.§

Adrenal cortical carcinoma, though rare, is the most frequent cause of Cushing's syndrome in infants and children. The finding of elevated plasma and urinary corticosteroids following ACTH stimulation should aid in the differentral diagnosis of adrenal cortical tumors.

Summary

The case of an infant with Cushing's syndrome due to adrenal cortical carcinoma

‖ Wilkins, L., personal communication with the authors.

¶ References 50 and 51.

References 52-57.

* References 63-65.

† References 66 and 67.

‡ References 66, 68-70.

§ References 71 and 72.

is presented. Symptoms in this patient began at the age of 6 weeks, making it the youngest patient with this disease reported to date. The fact that the mother of the infant had diabetes is of special interest.

Twenty-four cases of adrenal cortical carcinoma associated with Cushing's syndrome in children under 14 years of age are selected from the literature and tabulated.

Persistent elevations of plasma and urinary 17-hydroxycorticosteroids and urinary 17-ketosteroids after ACTH stimulation provide an additional aid for the diagnosis of adrenocortical carcinoma.

This patient was admitted on the service of Drs. C. Francis Scalessa and W. Dabney Jarman. Drs. E. Clarence Rice and Theodore Winship provided advice and criticism for this report. Edward Riggs supplied the histological preparations.

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RETROPHARYNGEAL ABSCESS

Presentation of a New Syndrome in an Unusual Case

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THE RETROPHARYNGEAL space is subject to bacterial invasion from the nasopharynx and from the cervical vertebrae, each mode of invasion giving its own characteristic clinical picture. Since the advent of chemotherapy, abscesses of the retropharyngeal space have become rare, but in recent case reports * the clinical picture is the same as described in the last century.†

As pyogenic organisms acquire resistance to therapeutic agents, the several complications of nasopharyngitis once controlled are reoccurring. Such diseases as mastoiditis and peritonsillar as well as retropharyngeal abscess are seen with increasing frequency and often present new or unusual signs and symptoms.

The case reported herein presents a new syndrome associated with retropharyngeal abscess.

Report Of a Case

A 5-year-old girl of Mediterranean origin was first seen on March 26, 1954, with a chief complaint of a stiff neck of one month's duration.

The present illness began on Feb. 25, 1954, when she was taken to a physician because of a sore throat, "stiff neck" (limited lateral movement of the head, head held in a semiflexed position, and inability to extend the head to the neutral position), and cervical adenitis presumably of the anterior cervical chain. She received 300,000 units of procaine penicillin G on each of two successive days with a concurrent subsidence of temperature and pharyngeal symptoms, but the "stiff neck" remained. Symptomatic therapy for this complaint was prescribed, and on one occasion she was taken to a chiropractor whose manipulations failed to relieve the patient.

On March 12, 1954, she complained of inability to swallow solid food. On March 24, 1954, she was taken to a local pediatric clinic where the resident, presuming this to be a cervical subluxation,* referred the patient to the author. Roentgenograms of the cervical region (Fig. 1) were taken at this time, and the child was admitted to the Rochester General Hospital on March 26, 1954.

Fig. 1.—March 26, 1954, roentgenogram on admission demonstrating the large retropharyngeal mass, loss of normal cervical lordosis, and "subluxation" of C-2 on C-3.

Physical examination on admission revealed a chronically ill 5-year-old girl in no acute distress. She was unable to swallow solid food. Her neck

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* References 1, 2 and 3.

† References 4 and 5.

was held semiflexed. Flexion of the neck was painless but extension even to the neutral position was not possible. Pain also limited lateral movement of the head to 15 degrees in either direction. A tender gibbus posteriorly at the level of C-2 was present. Thus complete examination of the oronasopharynx was not possible, but with this limitation the remainder of the physical examination was normal. The temperature, pulse, and respiratory rate were normal.

Laboratory Data.—The hemoglobin was 13 gm/100 cc., and the white blood cell count was 35,000, with 80% polymorphonuclear leucocytes, 18% lymphocytes, 2 % monocytes. A nose and throat culture showed Staphylococcus aureus and some pneumococci. An x-ray film taken on admission (Fig. 1) revealed the presence of a large retropharyngeal mass, obliteration of the normal cervical lordosis, and subluxation of C-2 on C-3.

After consultation with the anesthesia and otolaryngological service,‡ surgery without anesthesia was recommended. Laryngoscopy carefully performed revealed the apex of the retropharyngeal mass to be yellow in color, fluctuant, and directly opposite the glottis. The incision was painless and approximately 30 cc. of thick yellow pus under tension was recovered.

Fig. 2.—Roentgenogram March 31, 1954, 48 hours after incision and drainage of the abscess. The cervical derangement previously described (Fig. 1) is less evident and the retropharyngeal space is smaller in size.

‡ Dr. George Trainor, otolaryngologist, incised the retropharyngeal abscess.

Fig. 3.—Roentgenogram in June, 1955, showing normal anatomical relationship of the cervical elements.

After surgery the temperature rose to 103 F. Penicillin having been started prior to operation, no further therapeutic measures were deemed necessary. The temperature became normal 12 hours later. The remainder of the hospital course was uneventful.

Culture of the abscess revealed a predominence of beta hemolytic Streptococcus, a few pneumococci, and occasional Staphylococcus albus hemolyticus. On the second postoperative day, she was able to move her neck with minimal evidence of previous limitations and a roentgenogram (Fig. 2) showed marked reduction in size of the retropharyngeal space.

A roentgenogram one year later (Fig. 3) showed complete restoration of normal anatomic relationship of cervical elements and no evidence of scar or abnormality of the cervical vertebrae.

She has not been ill since this episode, in particular there has been no recurrence of nasopharyngeal or parapharyngeal pathology.

Comment

Retropharyngeal abscess is representative of the many sequelae to nasopharyngitis, which have become rare since the advent of chemotherapy and antibiotics. During this era physicians have become unfamiliar with these problems so that when such diseases now occur they either meet an inexperienced physician or, under the influence of chemotherapy ("adequate" or "inadequate"), present signs and symptoms bearing little resemblance to descriptions once considered "characteristic."

Descriptions of the symptomatology of retropharyngeal abscess in pediatric textbooks have not changed from 1890 § to recent times.‖

Infections in the nasopharynx may spread to the retropharyngeal space by way of lymph channels causing lymphadenitis, suppuration, and abscess formation. The retropharyngeal lymph glands are usually four to six in number, paired on either side of the midline, and separated by the buccopharyngeal fascia. Thus retropharyngeal abscesses secondary to nasopharyngeal infections are localized to one side of the midline. Since these retropharyngeal glands disappear during the second and third year of life, retropharyngeal abscess is characteristically a disease of infancy and "almost never seen after the age of three." [7]

Retropharyngeal abscesses usually rupture into the oropharynx with subsequent granulation and healing of the abscess cavity but, if untreated, present a mortality rate of 10%. Death may be caused by dissection of the abscess into the mediastinum, pneumonia, or rupture of the abscess in such a manner as to asphyxiate [2] the patient.

Bacteriologic studies have shown the infections to be due to a mixed flora in 60% of the cases, but hemolytic streptococci are usually found to predominate. Where pure cultures are obtained, again hemolytic streptococci predominate. This pattern has been true in all respects over the past 50 years and has not been changed by the advent of chemotherapy. This case, therefore, follows established rules.

In contrast to this source of retropharyngeal infection, invasion may come from the cervical vertebrae. This form of abscess is usually chronic, tuberculous foci being found in the cervical vertebrae. This chronic form of invasion apparently destroys the buccopharyngeal fascia so that the abscess comes to lie in the midline.

The symptomatology of retropharyngeal infection is due to the presence of an infection (i.e., nasopharyngitis with fever and toxic symptoms depending on the invading organism) and a mass behind the pharynx. This mass pushes forward, ultimately interfering with deglutition and respirations, depending upon the location of the abscess. If the apex of the abscess is located in the nasopharynx, interference with nasal respirations is suffered, but, if the apex is opposite the glottis, interference with deglutition and respiration is maximal and may be serious. In order to assure an adequate airway under such circumstances the head is held in full extension with the mouth open—a position considered by many to be pathognomic of this disease. The signs and symptoms of retropharyngeal invasion may appear within 48 hours of onset of the primary pathology or develop over a three- to four-week period.

The case reported herein is unusual in many respects. The chronicity of the course and the age of the patient are prominent exceptions to standard descriptions. Of greater importance, however, is the clinical syndrome evolved in this case, perhaps influenced by chemotherapy and as such an indication of the future syptomatology of this disease.

In 1952, Townsend and Rowe [8] studying the mobility of the cervical spine found a considerable degree of mobility of C-2 on C-3 present in all children. They also found that pharyngitis and allied disorders might be accompanied by a forward displacement of C-2 on C-3. They postulated that retro-

§ References 4 and 5.

‖ References 6 and 7.

pharyngitis and consequent spasm of the retropharyngeal muscles were etiologic factors in the pathogenesis of the syndrome. This case proves such a postulate to be tenable. One might reconstruct the pathogenesis of the presenting syndrome in this case in the following manner: During a pharyngeal infection, bacteria migrated via the lymph channels causing retropharyngeal cellulitis with consequent spasm of anterior paraspinal muscles. Initially this case might have shown a roentgenogram similar to those previously reported.[8]

Chemotherapy and antibiotics subdued any overt manifestation of retropharyngeal abscess formation which thus could develop slowly presenting a "chronic" type invasion with destruction of the buccopharyngeal fascia so as to allow central placement of the abscess. The cervical region, initially caught in this position of anterior flexion, remained flexed with subluxation of C-2 on C-3 while the abscess developed and increased in size.

Rapid and complete reversal of the clinical syndrome upon drainage of the abscess is presumptive evidence that no other pathology was influencing the clinical picture in this area.

Thus, a clinical syndrome unchanged in standard description over fifty years may, under the influence of chemotherapy and antibiotics whether adequate or inadequate, assume new facets which outwardly seem incompatible with the supposed pathology. The application of other pathogenetic processes, however, may serve to explain the apparent reversal of a clinical syndrome.

Summary

A case of retropharyngeal abscess in an older child presenting a new clinical syndrome has been reported. The pathogenesis of this syndrome is discussed.

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Abstracts from Current Literature

American Heart Journal, St. Louis
51 :647-806 (May) 1956

*An Ontogenetic Theory for the Explanation of Congenital Malformations Involving the Truncus and Conus. Maria V. De La Cruz and Jose Pio Da Rocha.—p. 782.

An Ontogenetic Theory for the Explanation of Congenital Malformations Involving the Truncus and Conus.—This article does not lend itself to satisfactory abstraction and should be read *in toto*.

Based on the embryological work of Davis, Streeter, and Kramer on a study of embryos in the Carnegie Collection, and on a study of clinical material, the authors have developed an ontogenetic theory to explain congenital truncoconal malformations. These malformations are believed to result from developmental alterations of the conoventricular flange, the truncoconal ridges, and the primordia of the aortic and pulmonary cusps. Their theory may be applied to abnormalities involving lateral positions of the great vessels, transposition, unequal partitioning of the truncus at the expense of the aorta or pulmonary artery, common truncus and valvular defects. The authors apply their theory in particular to some of the more commonly encountered congenital malformations, including common truncus, Eisenmenger complex, tetralogy of Fallot, solitary aortic trunk, tranposition of the great vessels, and the Taussig-Bing complex.

American Review of Tuberculosis, New York
73:157-313 (Feb.) 1956

*Lymph Node Tuberculosis in Neck, Axilla, and Groin. Charles W. Lester and Julia M. Jones.—p. 229.

Calcification of Regional Lymph Nodes Following BCG Vaccination. Samuel C. Stein and Martin J. Sokoloff.—p. 239.

Virulence for Guinea Pigs of Tubercle Bacilli Isolated from Pulmonary Lesions Resected During Chemotherapy. Joseph P. Kazlowske, Edwin Brosbe, James W. Raleigh, and Sidney Bernstein.—p. 266.

Fatal Case of Tuberculosis Produced by BCG. H. G. Calwell.—p. 301.

Antimicrobial Treatment of Primary Tuberculous Infection in Children. Stefan Grzybowski.—p. 301.

73:315-450 (March) 1956

Experimental Air-Borne Infection: A Comparison of Different Masks in the Protection of Rabbits Against Inhalation Infection with Tubercle Bacilli. Samuel Abramson.—p. 315.

Lobar Ventilation in Man. C. J. Martin and A. C. Young.—p. 330.

*Tuberculosis in Infants and Children: A Review. John S. Chapman.—p. 422.

73:451-608 (April) 1956.

Coccidioidomycosis: A Review and Presentation of 100 Consecutively Hospitalized Patients. Denis J. O'Leary and Francis J. Curry.—p. 501.

The Reflex Responses to Inflation or Deflation of the Lungs and Their Importance in the Regulation of Respiration. J. Garcia Ramos.—p. 519.

*Cycloserine: Antituberculous Activity in Vitro and in the Experimental Animal. William Steenken Jr., and Emanuel Wolinsky.—p. 539.

The Significance of Antibodies to Tuberculoprotein and Polysaccharide in Resistance to Tuberculosis: Interference with Antibodies by These Antigens. Florence B. Seibert, Elizabeth E. Miller, Ute Buseman, Mabel V. Seibert, Eva Soto-Figueroa, and Lois Fry.—p. 547.

Serologic Investigations of Mycobacterial Antigens: I. The Specificities of Aqueous and Saline Extracts of BCG, Mycobacterium Phlei, and H37Rv. J. R. Thurston, M. Rheins, and T. Huziwara.—p. 563.

Serologic Investigations of Mycobacterial Antigens: II. Specificities of Antigens Obtained by Successive Aqueous Extraction of BCG and Mycobacterium Phlei. J. R. Thurston and M. Rheins.—p. 571.

Lymph Node Tuberculosis in Neck, Axilla, and Groin.—The treatment of tuberculosis of the lymph nodes is discussed with reference to 66 cases since the advent of chemotherapy. These patients were mostly adults. Medical therapy alone was used in 12 cases and combined medical and surgical therapy in 54. Surgical therapy consisted of removal of all diseased nodes and excision of abscesses when present. If the abscesses were large, drainage and instillation of streptokinase-streptodornase solution was done as a preliminary measure. The authors stress the need of general supportive care and antitubercular drug therapy during the acute stage until the lesions become stationary. At that point they feel that surgery should be carried out to eradicate the lesion.

Tuberculosis in Infants and Children, A Review.—One hundred and one tuberculous patients, discharged from a children's hospital, are reviewed. Fifty-six per cent were under the age of three years.

Possibility of multiple sites of invasion is suggested by widespread distribution of disease in both lungs in 20% of cases and occasional occurrence of tuberculosis imbedded within the tonsils.

Lack of real differentiation from tuberculosis as seen in adults is indicated by the repeated occurrence of pulmonary necrosis in the presumed sites of invasion, development of superficial implantation type of bronchitis, and occurrence of effusions.

The author notes the recent adoption of a basic drug regimen for all cases. This includes isoniazid, 4 mg/kg., and PAS, 200 mg/kg. per day, with both drugs administered for a minimum of one year. In children with miliary or meningeal infection isoniazid dosage has been increased to 10 mg/kg. for the first two weeks with the addition of streptomycin-PAS for the entire course.

Treatment of tuberculosis of the superficial lymph nodes includes isoniazid-PAS, excision of the nodes when practicable, incision and drainage when the nodes are fluctuant, and the application of radiation therapy when the nodes are multiple or when palpable masses persist after drainage.

Evidence is offered which suggests that adequate drug therapy of tuberculous osteoarthritis may allow less drastic surgical management.

Cycloserine: Antituberculous Activity in Vitro and in the Experimental Animal.—Studies revealed that cycloserine inhibited the growth of human virulent tubercle bacilli in vitro in concentrations of 6.25γ per ml. in liquid medium and 20γ in solid medium. Although cycloserine was relatively ineffective in the therapy of experimental tuberculosis of mice and guinea pigs, other studies have noted that this drug produced a definite, but limited, beneficial effect in experimental tuberculosis of rabbits and monkeys. This situation demonstrates the importance of using several animal species for the evaluation of potential antituberculous agents.

Journal of the American Medical Association, Chicago

160:819-922 (March 10) 1956

*Fibrocystic Disease of the Pancreas, a Generalized Disease of Exocrine Glands. Paul A. di Sant'Agnese.—p. 846.

160:923-1004 (March 17) 1956

*Clinical Investigation on Attenuated Strains of Poliomyelitis Virus: Use as a Method of Immunization of Children with Living Virus. H. Koprowski, T. W. Norton, G. A. Jervis, T. L. Nelson, D. L. Chadwick, and D. L. Nelson.—p. 954.

*Ventricular Septal Defects with Pulmonary Hypertension: Surgical Treatment by Means of a Mechanical Pump-Oxygenator. J. W. DuShane, J. W. Kirklin, R. T. Patrick, D. E. Donald, H. R. Terry, and H. B. Burchell.—p. 950.

160:1005-1100 (March 24) 1956

*The Pediatrician Examines the Mother and Child: Introducing a Chart to Record Growth and Development. E. Robbins Kimball.—p. 1033.

160:1439-1538 (April 28) 1956

Management of Alkali Burns of the Eosophagus. G. M. Carver Jr., W. C. Sealy, and M. L. Dillon Jr.—p. 1447.

*Natural Course of Active Rheumatic Carditis and Evaluation of Hormone Therapy. M. G. Wilson and Wan Ngo Lim.—p. 1457.

161:1-120 (May 5) 1956

Grid Technique as a Tool for Improving Health Services to Institutionalized Children. Ralph Fried and M. F. Mayer.—p. 1.

*Salient Points in the Clinical Diagnosis of Congenital Heart Disease. B. M. Gasul and E. H. Feil.—p. 39.

Adenomas of the Rectum and Colon in Juvenile Patients. Robert Turell and A. de L. Maynard.—p. 57.

161:121-182 (May 12) 1956

*Complement Fixation in Cat Scratch Disease Employing Lygranum C. F. as Antigen. Charles Armstrong, Worth B. Daniels, Frank G. MacMurray, and Horace C. Turner.—p. 149.

161:183-302 (May 19) 1956

Toxoplasmosis In Man and Swine—An Investigation of the Possible Relationship; David Weinman and A. H. Chandler.—p. 229.

Fibrocystic Disease of the Pancreas, a Generalized Disease of Exocrine Glands.—The author presents a new concept of the nature of fibrocystic disease of the pancreas. This concept has evolved during the past few years with the demonstration of the consistent occurrence of a specific abnormality of sweat in patients with this disease. The various systems envolved and the resulting symptoms are discussed.

In the author's series of 325 patients the pancreas was involved in all but 10% and the respiratory tract in nearly all patients. Respiratory disease accounted for 90% of the deaths. "Focal biliary fibrosis" was found at autopsy in 25% of 116 autopsied patients. In 7 of the 325 patients the syndrome of portal hypertension was present. The author states that fibrocystic disease of the pancreas accounts for one-third of patients with this symptom complex in the pediatric age group. Sweat chloride and sodium are increased two to four times above the concentration found in normal persons. This abnormality is found in 99% of patients with this disease, and the determination is a valuable diagnostic tool. Abnormalities in the secretion of the salivary glands and duodenal contents are discussed. A section is devoted to the diagnosis, prognosis, and treatment of the disease.

Clinical Investigations on Attenuated Strains of Poliomyelitis Virus: Use as a Method of Immunization of Children with Living Virus.—The authors present a detailed description of their investigations concerning the problem of whether man can successfully be immunized against poliomyelitis by oral administration of live attenuated viruses. Under carefully controlled conditions 225 patients in a state mental institute received the poliomyelitis virus orally for immunization purposes. There were 150 patients who received the TN (Type 2) strain and 75 who received the SM (Type 1) strain. None of the subjects, as far as could be ascertained, suffered any ill-effect from ingesting the live virus. All developed antibodies. Attempts to suppress viral excretion in the stools by the use of drugs met with little success. Administration of gamma globulin at the time of virus feeding had no effect on antibody response. The authors conclude that with slow and careful research the living attenuated viruses may find their proper place in the line of combat against poliomyelitis.

Ventricular Septal Defects with Pulmonary Hypertension: Surgical Treatment by Means of a Mechanical Pump-Oxygenator.—The authors report an open intracardiac technique to close ventricular septal defects using a mechanical pump-oxygenator. There were 20 patients operated on using this technique between March and October 1955. Of these 20 patients 19 were under 12 years of age. All but two of the children were underweight and moderately to seriously handicapped with regard to exercise tolerance. In 3 patients the defect was closed by direct suturing, whereas in 17 patients a nonabsorbable polyvinyl sponge was placed in the opening and sutured to the rim of the septal defect. Sixteen patients survived operation and have shown pronounced improvement. Laboratory procedures used to determine the physiopathology of the heart before, during, and after surgery are briefly discussed.

The Pediatrician Examines the Mother and Child: Introducing a Chart to Record Growth and Development.—The fact that there was considerable variation in response of children to test situations prompted a study to determine the causes. A 10-year survey of testing the mental development of children at the Evanston Infant Welfare Society revealed that the reactions were affected not only by the presence of the mother but also by the personalities of the parents when neither were present. Other factors which seemed to affect adaptability to test situations were the sex, the place in the family, breast feeding, and the amount of compartmentation (separation of child play from adult dangers) practiced by the parents. The author introduces a chart on which are recorded not only the physical and mental development but also the various factors affecting the child's adaptability. One of the conclusions from this study was that the use of compartmentation increased the adaptability of the school child more than twofold and that of the toddler nearly fourfold.

Natural Course of Active Rheumatic Carditis and Evaluation of Hormone Therapy.—In reviewing the course of 100 patients who experienced one or more attacks of rheumatic carditis and were treated symptomatically, the authors found that the severity and duration of rheumatic carditis remained remarkably constant in the span of years from 1930 to 1955. From 1949 to 1955, fifty-five patients with active carditis of 3 to 21 days duration were given hormone therapy. The hormones used included corticotropin (ACTH), cortisone (compound E), and hydocortisone (compound F). The average duration of therapy was seven days.

The results of the hormone-treated group of patients revealed that the duration of active carditis was less than one month in 69% and less than two months in 31%. In 84% of the patients treated, whose attacks were initial, there was no overt evidence of residual cardiac damage. The course of carditis in patients receiving hormone therapy

was in marked contrast to that of the control group both in duration and extent of residual cardiac damage.

Salient Points in the Clinical Diagnosis of Congenital Heart Disease.—The authors review the clinical and laboratory findings of 1395 patients with congenital heart disease. In their opinion in the vast majority of patients a clinical diagnosis can be made without angiocardiography and without cardiac catheterization. The incidence of the various types of heart disease in this series is given. Twenty of the commoner types of malformations are discussed in some detail with the salient points of diagnosis presented. In summarizing the findings of the cyanotic patients, the authors state that if the heart presents roentgenologic and fluoroscopic evidences of not being definitely enlarged and pulmonary vascular markings that are diminished the patient most likely has a malformation of the heart amenable to surgery by means of a Blalock-Taussig, Potts-Smith, or a Brock pulmonary valvulotomy.

If, on the other hand, the cyanotic patient presents roentgenologic and fluoroscopic findings of a definitely enlarged heart and, even more important, if the pulmonary vascular pulsations and markings are definitely increased, surgical intervention with the above-mentioned operations is almost never possible.

Complement Fixation in Cat Scratch Disease Employing Lygranum C.F. as Antigen.—In view of reports dealing with postive complement fixation reactions in patients with cat-scratch disease using the Lygranum C.F. antigen the authors examined by the same technique serums from 71 persons who had no known attack of cat-scratch disease and found 8 positive reactors (11.2%). There were 2 (5%) positive reactors among 40 of these controls whose ages varied from 4 to 25 years. There were six positive reactors in persons from 32 to 68 years of age.

In the authors opinion the demonstration of a positive complement fixation to Lygranum C.F. antigen is without diagnostic value in the individual case of cat-scratch disease and affords no convincing evidence that the disease belongs to the psittacosis-lymphogranuloma group of diseases.

Canadian Medical Association Journal, Montreal

74:601-678 (April 15) 1956

Infectious Mononucleosis. J. Lowney and G. X. Trimble.—p. 636.
A Case of Fatal Cyclic Vomiting. M. LeClair.—p. 641.
*Interstitial Plasmacellular (Parasitic) Pneumonia in Infants. F. Gagne and F. Hould.—p. 620.

74:678 (May 1) 1956

*Report of a Successful Operation for Stenosis of Common Pulmonary Vein (Cor Triatriatum). A. Vineberg and O. Gialloreto.—p. 719.
Agamma globulinaemia. J. A. O'Brien and M. M. Sereda.—p. 723.

Interstitial Plasmacellular (Parasitic) Pneumonia in Infants.—The authors report three cases of plasmacellular pneumonia in infants. The disease is considered to be quite common in Europe, but rare in America, and to be due to a parasite, Pneumocystis carinii. It should be suspected in premature or hypotrophic infants with respiratory distress. The parasites appear as minute dots or comma-like basophilic granules surrounded by a clear space and a thin, homogeneous, bluish capsule. The problems of taxonomy of Pneumocystis and its mode of propagation are discussed. Treatment is supportive and symptomatic, antibiotics being totally ineffective.

Report of a Successful Operation for Stenosis of Common Pulmonary Vein (Cor Triatriatum).—Stenosis of the common pulmonary vein is very rare. Three types of such abnormality are mentioned: (a) with wide connection between the accessory chamber and the left atrium, (b) one or several small openings between the accessory chamber and the atrium, and (c) no connection between these chambers. Details of such a case, successfully operated upon, are given with illustrations and diagrams. The clinical picture resembled that of mitral stenosis.

Acute Contagious Diseases

THE EPIDEMIC INCREASE OF POLIOMYELITIS IN THE NETHERLANDS AND ADJACENT COUNTRIES IN 1952. J. J. VAN LOGHEM. Nederl. tijdschr. geneesk. 99:183 (Jan. 15) 1955.

During the summer of 1952, an epidemic of poliomyelitis occurred in a large part of Western Europe. A total of 32,000 cases was registered. A statistical analysis of this epidemic is made with the help of the public health authorities of the districts and provinces involved.

It appeared that the nuclear region of this epidemic was localized in Western Germany, Eastern Holland, and Northeastern Belgium. In the surrounding districts the epidemic developed later, the peak being on an average lower than in the nuclear region. It is pointed out that in the nuclear region, which neither sociographically nor geographically is a unit, the epidemic was characterized by synchronism and isomorbidity.

VAN CREVELD, Amsterdam.

Acute Contagious Diseases

RESULTS OBTAINED WITH DIPHTHERIA-PHOSPHATE-TOXOID. A. TASMAN and J. D. LEBRET, Nederl. tijdschr. geneesk. 99:1030 (April 2) 1955.

There were 4272 persons immunized with diphtheria-phosphate-toxoid. The number of unpleasant reactions was small in both children and adults. The Schick conversion rate was 83% after one injection and 99% after two injections; therefore it is advisable to give one or two booster injections to older children. The state of immunization of children under 6 years of age is bad in the district where this investigation was carried out (a small provincial town).

VAN CREVELD, Amsterdam.

Acute Infectious Diseases

CLINICAL RESULTS WITH THE SULFONAMIDE AMINDAN IN CHILDHOOD. HANS ALBRECTH, Med. Klin. 50:1830-1831, 1955.

Amindan is a compound of sulfanilamide and sulfosalicylic acid combined with sulfosalicylic-p-aminobenzolsulfonamid. It is readily soluble in water, the solution being strongly acid. A 3% solution has a pH of 2. Sodium bicarbonate 0.16 gm. is added to 0.5 gm. Amindan. The taste is pleasant and it is a good antipyretic. The dosage is stated in terms of tablets omitting the amount in the tablet. Amindan proved of benefit in various infections of infants and children.

SULPHONAMIDE THERAPY BY RECTUM IN CHILDREN. E. ORLANDO, Lattnate 26:647 (Oct.) 1955.

Observations with children of various ages revealed that the administration of suppositories containing mixed sulphonamides did not yield a satisfactory therapeutic blood level.

HIGGINS, Boston.

MENINGITIS IN THE NEWBORN. S. VAN CREVELD, Nederl. tijdschr. geneesk. 99:473 (Feb. 12) 1955.

In this clinical lecture the peculiarities of meningitis in the newborn and the difficulties in the recognition are stressed. Attacks of cyanosis with fever and vomiting occurring in a newborn without distinct etiology must suggest the possibility of meningitis. In other cases restless movements of the head, local muscle contractions, and screaming of the child give an indication for spinal puncture.

Early diagnosis and treatment are of the utmost importance for the recovery and for the future mental development of the child. Two cases are given as examples.

VAN CREVELD, Amsterdam.

RESULTS OF HETEROLOGOUS PLASMOTHERAPY IN CHILDREN. DR. R. RAMOS and W. OPPENHEIMER, Arch. pediat., Barcelona 4:537-551 (March-April) 1954.

The authors present the follow-up on 500 infants treated in a period of nine years with one or more infusions of desensitized calf plasma.

The types of illnesses and the number of infusions are tabulated. None suffered any allergic or anaphylactic reactions.

ALLEN, Santa Fe, N. Mex.

TREATMENT OF TETANUS IN THE NEWBORN AND THE CHILD. L. TORRES-MARTY and B. CASTELLANOS, Arch. pediat., Barcelona 5:503-509 (March-April) 1955.

Two cases of tetanus in a newborn and a boy of 8 are described in detail. In addition to the usual treatment recognized almost universally, the author advises artificial hibernation or hypothermia. Nasal feeding may also be a necessity. Antihistamines should be used to prevent allergic responses to tetanus antitoxin.

ALLEN, Santa Fe, N. Mex.

POLIOMYELITIS. JAMES P. NOLAN, BARBARA H. WILMER, and JOSEPH L. MELNICK, New England J. Med. 253:945 (Dec. 1) 1955.

The 1954 epidemic of poliomyelitis in New Canaan, Conn., a community enjoying a high standard of living, was studied with the aid of clinical, epidemiologic, and laboratory methods of investigation. The following aspects of the outbreak were examined: the epidemiologic setting, the number of patients and contacts with positive neutralization and complement fixation tests, the number of patients and contacts excreting virus, the correlation of clinical histories with the results of these laboratory studies, and an evaluation of the laboratory technics employed.

The initial focus of the epidemic was in a small nursery school, and infection was carried from here into the community by the school children predominantly to their families and intimate contacts of their families. Six of the sixteen cases were in adults between 27 and 35 years of age.

Serologic tests were performed on 106 persons. The complement-fixation test was positive for recent infection in 92% of the patients tested and in 40% of their contacts. Only in children under 6 years of age were predominantly monotypic responses obtained to the type of virus isolated in the stools. The neutralization test proved of little value as a diagnostic tool in this study, because the titers had usually reached high levels by the time the first sample was drawn.

Of 114 persons tested, virus was recovered from the gastrointestinal tract in 67. All virus obtained was Type 1, with the exception of three strains recovered from members of one family who were excreting a Type 2 strain. Virus was recovered from 88% of the patients and 54% of all contacts. Stool samples yielded virus about twice as often as rectal swabs obtained from the same persons. Virus was recovered twice as frequently from symptomatic contacts exhibiting minor illnesses as from those with no symptoms.

WHEELOCK, Washington, D. C.

DETERMINATION OF THE TYPE OF POLIOMYELITIS WITH COMPLEMENT FIXATION. VON W. HENNESSEN, W. JACOB and H. SEICHTER, Deutsche. med. Wchnschr. 80:1565-1566, 1955.

In a number of clinically diagnosed cases of poliomyelitis micro-complement-fixation tests were carried out. In 62.5% (of 364 patients) complement-fixing antibodies to Type I were found, in 27.6% to Type II, and in 9.9% to Type III.

REMARKS ABOUT THE SEVERE MENINGOCOCCEMIAS. W. O. SCHMITT, ALB. MEGEVAND, and S. SOROUCHYARI, Schweiz. med. Wchnschr. 85:1117-1120 (Nov. 12) 1955.

Attention is called to the peculiar course a meningococcus infection may present.

An 8-months-old girl had a fever. She groaned a little, there was loss of appetite, she vomited once and salivated. Other symptoms may prevail, respiratory, gastrointestinal, or arthralgia. This prodromic stage lasted about 12 hours. Petechiae appeared with this attack and continued to appear for three afebrile days. During this period a leucopenia existed. Then fever recurred with hyperleucocytosis. In the other two cases, a boy 15 months of age and a girl six and a half years of age, this prodromic stage was less clearly defined or absent. The importance of an early diagnosis is stressed; the authors recommend the examination of material from petechiae for meningococci, examination of spinal fluid and blood culture. The severity of the Waterhouse-Friderichsen disease may be judged by the lowered blood pressure which can be rectified by noradrenaline. Administration of penicillin and sulfonamides in large doses is recommended.

AMBERG, Rochester, Minn.

C REACTIVE PROTEIN IN THE BLOOD OF PATIENTS WITH ACUTE INFECTIOUS DISEASES. J. VAN DER VEEN and E. D. A. M. SCHRETLEN, Nederl. tijdschr. geneesk. 99:1217 (April 23) 1955.

In a preliminary investigation the occurrence of C reactive protein in the blood of patients with various acute infectious diseases is demonstrated. These findings are in accordance with the results previously described by others. When the pathological process is no longer active, the C reactive protein disappears from the blood. The available evidence seems to indicate that the presence of C reactive protein is probably as sensitive to the activity of infectious diseases as the erythrocyte sedimentation rate, but the results of the two methods did not run parallel.

In judging the reaction to C reactive protein, unlike the sedimentation rate, it is not necessary to reckon with results, which keep within the limits of possibility. The test for C reactive protein may be a valuable aid in determining the presence of inflammatory conditions and expressing the activity of pathological processes in a tentative figure. This may be valuable in cases of acute rheumatic conditions.

VAN CREVELD, Amsterdam

POSSIBILITY OF TRANSMISSION OF SERUM HEPATITIS VIRUS BY INTRACUTANEOUS INJECTION. A. CH. RUYS and J. PRAKKEN, Nederl. tijdschr. geneesk. 99:1163-1164 (April 16) 1955.

In mass intracutaneous injections, usually the same syringe is used a number of times without sterilizing, while for each person only the needle is changed. It is shown that by back-pressure from the site of injection, erythrocytes of the recipient may enter not only the needle, but also the syringe. It is advisable therefore to use a syringe only once between sterilizings. This is the only way in which to avoid the transmission of the virus of serum hepatitis.

VAN CREVELD, Amsterdam

CONGENITAL SKELETAL MALFORMATION DUE TO MATERNAL VIRAL HEPATITIS DURING PREGNANCY. E. DOLL, Monatsschr. Kinderh. 103:391-393 (Aug.) 1955.

Multiple congenital skeletal malformations occurred in an infant born to a mother who had developed viral hepatitis during the first trimester of her pregnancy.

Such malformations were reported in an infant whose mother contracted viral hepatitis two months prior to delivery.

HARTENSTEIN, Syracuse, N. Y.

Chronic Infectious Diseases

PROBLEMS IN THE USE OF ANTI-TUBERCULOSIS DRUGS IN INFANCY. A. CLARIANA, Arch. pediat., Barcelona 4:467-497 (March-April) 1954.

The author outlines the uses of PAS, streptomycin, and isonizide. He discusses the scheme of treatment in miliary tuberculosis and the various adenopathies depending on their anatomical location; also the type of pulmonary tuberculosis called infiltration.

He also describes the toxic reactions which may develop. The dosages according to age, length of duration of the infection, and the age of the patient are tabulated.

The usual clinical criteria and x-ray films are used to determine the process of cure. The duration of treatment varies, but in his resumé he says that in cases where a remission does not take place in the expected time a substitution of one drug for another or two together may help. Even if there are no toxic reactions, this association of two drugs at once or a change of the single drug brings an earlier cure.

ALLEN, Santa Fe, N. Mex.

TUBERCULOSIS IN TWINS. VERSCHUER and O. FRHR, Deutsche. med. Wchnschr. 80:1635-1637 (Nov. 11) 1955.

Identical twins showed a greater similarity in their response to tuberculous infection than fraternal twins. From the literature and observations of altogether 617 pairs of twins similar reactions to the infection were registered in 74% of identical against 28% in fraternal twins. Forty identical and sixty-two nonidentical twins could be checked after 20 years. Here also the reactions of the identical twins were more frequently similar than those of nonidentical twins, whereby the relatively slight differences between the first and later observations were not statistically significant. When only one of a pair of identical twins showed tuberculous lesions, the other twin died of tuberculosis only in 1 of 11 instances. Of 34 nonidentical twins, 19 of the primarily not infected twins died of tuberculosis. It is of further interest that the infected identical twins of this series often recovered. Aside from other constitutional factors a specific hereditary disposition toward tuberculosis is assumed.

AMBERG, Rochester, Minn.

PROGRESS AND PROBLEMS OF THE TREATMENT OF TUBERCULOSIS MENINGITIS IN CHILDREN. LOTHAR WEINGARTNER, Med. Klin. 50:1729-1734, 1955.

The improvement of the treatment of tuberculous meningitis is outlined on the basis of 226

patients from 1948-1954, referable to the introduction of various tuberculostatic drugs. Features of prognostic importance are discussed. The dosage of the remedies is outlined with attention to possible injuries, and the duration of therapy, streptomycin, isonicotinic acid hydrazid, para-aminosalicylic acid, and thiosemicarbazon and the mode of administration are considered. Treatment with isonicotinic acid hydrazid combined with streptomycin treatment has been adopted.

ON THE PROGNOSIS OF CHILDHOOD TUBERCULOSIS: AN 18 YEAR FOLLOW-UP REPORT. NILS LEVIN, Am. Rev. Tuberc. 72:513 (Oct.) 1955.

This study of 426 children followed for a period of 16 to 22 years was reported from Uttran, Sweden. All patients had received detailed x-ray and bacteriologic studies initially, and a 94% follow-up on x-ray was obtained as well as a complete follow-up on those who had died of the 254 children who had initially shown active tuberculosis 82% had no signs of active lesions on the follow-up x-ray study. Thirty-four children (13%) had died, twenty-six of lung complications and eight of tuberculous meningitis. The death rate of children who initially showed postprimary lesions was nine times that of those initially showing primary tuberculosis. But death from tuberculous meningitis was commoner in the group initially showing primary lesions. In the "primary" group the mortality rate increased with the age of the child at diagnosis; the reverse was noted in the postprimary group. Both groups had proportionately high death rates in the children who contracted tuberculosis during puberty.

ROYCE, Pasadena, Calif.

Diseases of Blood, Heart and Blood Vessels, and Spleen

BANTI'S DISEASE AND FIBROUS CONGESTIVE SPLENOPATHY IN CHILDREN. P. FORNARA and M. GENESI, Minerva pediat. 7:1357 (Nov. 3) 1955.

Splenectomy was performed on a boy of 8 years; the child died 5 years later of an infection following episodes of marked ascites. Of four cases of congestive splenomegaly in children a splenectomy was done in three; two of these three children died six and eight years later of internal hemorrhages. Microscopic sections of the liver and spleen of these children are pictured. In the case of Banti's disease, there was marked hepatic cirrhosis, and practically no sequellae of thrombosis found. In the cases of congestive splenomegaly hepatic cirrhosis was rare, but the sequellae of thrombosis are very evident. Congestion of the spleen was not present in the case of Banti's disease. Splenectomy was observed to have a beneficial effect in the cases of congestive splenomegaly; but it does not lower portal hypertension; if that condition exists, eventually severe hematemesis can occur. It is recommended that splenoportography and manometric measurements be made in considering the advisability of splenectomy.

HIGGINS, Boston.

THE AMOUNT OF PYRUVIC ACID IN THE BLOOD OF CHILDREN WITH CONGENITAL HEART PATHOLOGY. E. SEGAGNI and G. BONO, Minerva pediat. 7:989 (Aug. 25) 1955.

The pyruvic acid content was determined in the bloods of 10 normal children and of 30 children with congenital heart pathology, 20 of whom were cyanotic. The mean finding for the healthy child was 88 mg/100 cc., for the cyanotic heart case 67 mg/100 cc., and for the non-cyanotic heart case 71 mg/100 cc.

Possible explanations for this quite consistent low normal pyruvicemia in cardiac cases are considered. This finding may be associated with the hypoglycemia or the low proteinemia so commonly found in heart cases. It is also quite possible that a different mechanism for the catabolism of pyruvic acid may be called into play.

HIGGINS, Boston

THE ACTION OF CORTISONE ON THE CONCENTRATION OF SODIUM SALICYLATE IN THE BLOOD. G. GELLO and L. DELLA SANTA, Minerva pediat. 7:1456 (Nov. 10) 1955.

The salicylate content of the bloods of 15 afebrile rheumatic patients were determined 1, 2, 3, 6, 9, and 12 hours after the administration of sodium salicylate (0.12 to 0.18 gm/kg.) and sodium bicarbonate. The same determinations were made after 50 to 75 mg. cortisone were given in addition to the salicylate. In 8 of the 15 patients, the blood salicylates were higher when the cortisone was given; in no case were they lower. The conclusion is drawn that cortisone usually increases salicylemia, improves the tolerance for salicylates, and thus provides a beneficial

synergism in the treatment of rheumatic fever. It appears quite likely that the cortisone exerts an inhibitory action on the renal clearance of salicylates.

HIGGINS, Boston

CLINICAL STATISTICS REGARDING 112 CHILDREN WITH RHEUMATIC FEVER, SEGAGNI, MINERVA pediat. 7:1309 (Oct. 27) 1955.

One hundred twelve children with rheumatic disease were admitted to the wards of the Pediatric Clinic of the University of Turin during the years 1946 to 1954, accounting for 1.21% of the total admissions. The lowest incidence in any one year was 5 (.55%) in 1947, and the highest 27 (1.86%) in 1953. There was no indication that the disease was becoming more or less frequent now as compared with 9 years ago. The disease was more frequent in the colder months than in the warmer (63 and 49 cases). The mean age was 8 years, being the same for boys and girls; 53 of the cases were in boys and 59 in girls. There was a family history of the disease in 51 of the cases. The onset followed recent attacks of scarlet fever in 7 cases, of tonsillitis or sore throat in 26 cases, of other streptococcic infections in 14 cases, and influenza in 2 cases. Forty-four per cent of the children showed signs of carditis on the first rheumatic attack, and seventy per cent of those having had previous attacks. The diastolic murmur of aortic insufficiency was present in only three children. Seventeen of the children had chorea, and three erythema nodosum. The RBC was less than 3,500,000 in 51% of the cases. Treatment was by penicillin and the sulfonamide drugs, by salicylates, and in the severer cases by ACTH and cortisone; 31% recovered from their attack without cardiac damage; 7% died.

HIGGINS, Boston

THE MEASUREMENT OF THE CARDIAC VOLUME IN CHILDREN BY MEANS OF TRANSVERSE AXIAL STRATIGRAPHY. L. BUFFONI and A. DE MAESTRI, Minerva pediat. 7:638 (June 2) 1955.

After placing a grid, with wires 1 cm. apart, over the child's chest, anterior-posterior and transverse stratigraphic films were taken. Measurements on these films allow one to calculate the volume of the heart.

HIGGINS, Boston

THE TREATMENT OF HEART FAILURE BY LANATOSIDE C. G. GRANATA and L. PARENZAN, Minerva pediat. 7:1292 (Oct. 20) 1955.

Lanatoside C (Cedilanid), a glucoside from the Digitalis lanata, proved very efficacious in relieving heart decompensation in five children with rheumatic heart disease; three of the children had acute carditis. The relative efficacy of the drug is due to its rapid action and to its failure to become fixed and accumulate in the body. The drug is quite safe, providing one gauges the dose so as to take into consideration the accumulation of other digitalis preparation previously given.

HIGGINS, Boston

ELECTROPHORESIS ON PAPER OF THE HEMOGLOBIN IN THALASSEMIA, DREPANOCYTOSIS AND THALASSODREPANOCYTOSIS. I. GATTO and A. LA GRUTA, Minerva pediat. 7:618 (May 19) 1955.

By paper electrophoresis (Ph 9.2%, Na barbital 2.1%), the different types of hemoglobin migrate as negative ions at different rates. In this way it is possible to identify carriers of the traits of thalassemia and drepanocytosis.

HIGGINS, Boston

CARDIAC CATHETERIZATION AND TRANSVERSE AXIAL STRATIGRAPHY IN THE DIAGNOSIS OF AURICULAR SEPTAL DEFECTS (WITH FEW SYMPTOMS) AND OF LUTEMBACHER'S SYNDROME. R. BULGARELLI, Minerva pediat. 7:1277 (Oct. 20) 1955.

The work-up of two cases of patent auricular septum is described, and the findings by x-ray, stratigraphy, and cardiac catherization well illustrated.

One case was of an 11-year-old boy who had a slightly enlarged heart, a protosystolic murmur over the body of the heart, and practically no symptoms. Among other findings, it was established that the blood in the right auricle had a lower CO_2 content than either the venous blood

or the right ventricle. Enlargement of the right auricle was shown on a film taken at the time the patient was swallowing a barium meal; there was apparent pressure of the auricle on the esophagus.

The other case was in a man of 41 years old with Lutembacher's syndrome. In this syndrome, there is a patent interventricular septum and a mitral stenosis, either congenital or acquired.

HIGGINS, Boston

COMPARATIVE ELECTROPHORETIC STUDIES OF THE PROTEIN CONTENTS IN THE VENOUS CIRCULATION AND IN THE MEDULLARY BLOOD OF NORMAL CHILDREN. Minerva pediat. 7:606 (May 19) 1955; A COMPARATIVE STUDY OF THE PROTEIN CONSTITUENTS OF THE PERIPHERAL AND MEDULLARY BLOODS OF CHILDREN WITH VARIOUS DISEASES. W. TANGHERONI AND R. BARTALENA, Minerva pediat. 7:1300 (Oct. 20) 1955.

These two papers present the differences found in the protein constituents of the peripheral and the medullary blood of normal children and the changes found in certain illnesses. The medullary blood was obtained by needle puncture of the tibia, the ilium or, the sternum; about 0.5 cc. was withdrawn, as only 0.01 cc. of serum was needed for the electrophoretic study. The only consistent differences between the two bloods were in the albumin content, the A/G ratio, and the gamma-globulin content.

In the well child, the albumin and the gamma-globulin contents were greater in the medullary than in the peripheral blood; the A/G ratio also usually was higher. Deviation from this finding were found in various diseases. The gamma-globulin content of the medullary blood was markedly higher in the cases of children with acute gastroenteritis, hemolytic anemia, purpura, and dystrophy. The gamma-globulin content was definitely lower in the medullary blood of children with acute rheumatic fever, and in secondary and aplastic anemia. No differences were found in the protein contents of the medullary and peripheral bloods of children with nephritis, nephrosis, catarrhal jaundice, and pulmonary tuberculosis. These findings seem to indicate that the gamma-globulin may be formed in the bone marrow.

HIGGINS, Boston

THE SEASONAL INCIDENCE OF ACUTE LEUKEMIA IN CHILDREN. B. FANTUZZI, Minerva pediat. 7:604 (May 19) 1955.

The times of onset of 38 of the 50 cases of acute leukemia, seen at the Pediatric Clinic in Milan, were in the fall and winter months. This finding suggests that the disease has a seasonal incidence.

HIGGINS, Boston

THE URINARY EXCRETION OF THE 11-OXYCORTICOIDS AND THE 17-KETOSTEROIDS AND THE SERUM CONTENTS OF POTASSIUM AND SODIUM IN CHILDREN WITH ACUTE RHEUMATIC INFECTIONS UNDER TREATMENT WITH SODIUM SALICYLATE. M. LATO and A. CECCARELLI, Clin. pediat. 37:602 (Aug.) 1955.

When 23 children with acute rheumatic fever were given sodium salicylate, there was a definite and marked increase in the urinary excretion of the 11-oxycorticosteroids in each case; in 6 cases there was also an increase in the excretion of the 17-ketosteroids, but not to a degree above the physiological level; there were no significant or consistent changes in the amounts of potassium or of sodium in the blood serum. From these data, it appears that sodium salicylate has a pharmalogical and medicinal action on the adrenal cortex.

HIGGINS, Boston

CHRONIC PERICARDITIS. J. M. DURAN ANDREU, Arch. pediat. 4:481-504 (March-April) 1954.

This is a presentation of a chronic pericarditis in a child of 2½ years. The etiology of this chronic constrictive type of pericarditis was tuberculous. After antituberculosis treatment, surgical intervention was successful and all symptoms disappeared, including the specific electrocardiographic readings and x-rays.

The symptoms, diagnosis, details of the operation, and before and after photographs are included.

ALLEN, Santa Fe, N. Mex.

TEMPORARY GENERALIZED OEDEMA OF OBSCURE ORIGIN. R. S. ILLINGWORTH and E. FINCH, Arch. Dis. Childhood 29:513 (Dec.) 1954.

Two cases of edema with hypoproteinemia are described, one lasting for 5 weeks and the other for 14 months. Recovery in both cases was spontaneous and complete.

It is suggested that the cause in both cases lay in a temporary disturbance of protein metabolism of unknown origin.

KELLY, Milwaukee

CHRONIC GENERALIZED OEDEMA AND HYPOPROTEINAEMIA OF OBSCURE ORIGIN. R. S. ILLINGWORTH and E. FINCH, Arch. Dis. Childhood 29:507 (Dec.) 1954.

After a review of the literature, two cases of obscure generalized edema with hypoproteinemia and steatorrhea are described. One child died, and was found to have fatty infiltration of the pancreas and atrophic acini, without cystic fibrosis. The other child does not appear to have either cystic fibrosis of the pancreas or celiac disease. The cause of the hypoproteinemia was not determined.

KELLY, Milwaukee

ACUTE VASCULAR (SCHONLEIN-HENOCH) PURPURA—AN IMMUNOLOGIC DISEASE? MARSHALL B. KREIDBERG, WILLIAM DAMESHEK, and ROCCO LATORRACA, New England J. Med. 253: 1014 (Dec. 8) 1955.

The authors studied 15 children from 2 to 10 years of age who were admitted to the Boston Floating Hospital because of purpura. A full hematologic work-up was done in most instances.

The classic clinical picture of urticaria, palpable purpura, arthralgia, fever, and malaise is described in detail. The association of hemorrhagic glomerulonephritis with anaphylactoid purpura is common, and hematuria should be assiduously sought for during the course of the hematologic phenomena.

Etiologic theories (based on antigen-antibody mechanisms), the histologic studies, and the use of ACTH in treatment are all discussed.

The authors add the weight of their opinion to the thesis now commonly held that anaphylactoid purpura belongs to the "collagen" group of diseases which are probably initiated by beta streptococcal infection.

WHEELOCK, Washington, D. C.

THE PRODUCTION OF THROMBOPLASTIN IN THE BLOOD OF NEWBORNS. S. VAN CREVELD, H. BAKER, T. NIESSING, J. J. SIPKEMA, and C. A. A. M. SMITS, Nederl. tijdschr. geneesk. 99:1146 (April 16) 1955.

Quantitative and (or) qualitative changes in the platelets could not be found responsible for the abnormal course of the thromboplastin generation test (T.G.T.) found in premature newborn infants.

An isolated fraction of the plasma-thromboplastin component (P.T.C.) produced a change toward normal in the T.G.T., performed with normal plasma, normal platelets, and serum of premature infants. This speaks in favor of the presence of a P.T.C. deficiency in newborn premature infants. A normal level of the P.T.C. in premature infants was reached between the 21st and 25th day of life.

VAN CREVELD, Amsterdam

News and Comment

ANNOUNCEMENTS

Residency in Pediatric Oncology.—Applications for residency from July 1, 1957, to June 30, 1958, in Pediatric Oncology at Memorial Center for Cancer and Allied Diseases in New York will be received up to Nov. 1, 1956. This residency is approved for partial credit by the American Board of Pediatrics and is limited to the neoplastic diseases of children. Salary: $3600 annually plus maintenance. For application forms and information write: Director, Pediatric Service, Memorial Center for Cancer and Allied Diseases, 444 East 68th Street, New York 21.

Postgraduate Course in Pediatric Allergy.—New York Medical College, Flower and Fifth Avenue hospitals, Division of Graduate Studies, Department of Graduate Pediatrics announces a postgraduate course in pediatric allergy under the direction of Bret Ratner, M.D., Professor of Clinical Pediatrics and Associate Professor of Immunology. There will be 30 sessions, one each Wednesday, from 9 a.m. to 4 p.m., beginning on Nov. 7, 1956, and ending May 29, 1957; the fee is $300.00.

The course consists of lecture-seminars, laboratory and clinical procedures, clinic work, ward rounds, and animal experimentation covering basic principles of diagnosis and treatment of allergy in children, and applied immunology.

Applicants must be certified in pediatrics or have the requirements for certification. Enrollment is limited.

Applicatons should be sent to the Office of the Dean, New York Medical College, 5th Ave. at 106th St., New York 29.

A Research Fellowship in Pediatric Allergy is available; application should be made now.

GENERAL NEWS

Specialists Certified by the American Board of Pediatrics, Inc.—The following physicians were certified as specialists by the American Board of Pediatrics, Inc., in Atlantic City, N. J., on May 3, 4, and 5, 1956.

Agustsson, Hreidar, Aberdeen Proving Ground, Md.
Appleby, Robert Earle, Wilton, Conn.
Beasley, Albert Sidney, Westport, Conn.
Becker, Frederick B., Portsmouth, Va.
Bennett, Doris Ruth Rubin, Malden, Mass.
Blatman, Saul, Manhasset, Long Island, N. Y.
Brady, Joan Veronica, Urbana, Ill.
Cecil, Henry Shuford, Paoli, Pa.
Chambliss, Harriette Clark, Washington, D. C.
Chao, Dora Hsi-chih, Houston, Texas
Charash, Leon Irwin, New York.
Coffin, Grange S., Baltimore.
Cooper, Richard, San Antonio, Texas
Corbo, Paschal Anthony, Staten Island, N. Y.
Costeff, Hanan, Woodside, N. Y.
Cypress, George Christopher, Jr., Washington, D. C.
Deitch, Selma R., Needham, Mass.
Doe, Thomas A., Springfield, Mass.
Fellers, Francis Xavier, Amherst, Mass.
Field, William Grant, Ocean City, N. J.
Fisher, Marion White, Sandston, Va.
Gaskins, Albert L., Jr., Philadelphia.
Gersh, Marvin James, Tarrytown, N. Y.
Gershman, Martin, Fort Huachuca, Ariz.
Glass, Herbert Lee, New York.
Glazier, William, Winthrop, Mass.
Gonzalez, Romulo F., Syracuse, N. Y.
Gonzalez, Etervina Figueroa de, Santurce, Puerto Rico.
Gonzalez Alvarez, Osvaldo, Santurce, Puerto Rico.
Goren, Stanley Edwin, Philadelphia.
Guilbert, Paul William, Montreal, Canada.
Haggerty, Robert Johns, Boston.
Hearne, Calvin B., Wilmington, Del.
Hoekelman, Robert Arnold, Jr., Canandaigua, N. Y.
Jalbert, Eugene O., Manchester, N. H.
James, Grace M., Louisville, Ky.
Kitch, Charles D., La Mesa, Calif.
Kugler, Margaret M., Riverdale, N. Y.
Lechaux, Pierre Aymar, Washington, D. C.
Lee, J. Frederick, Greenwich, Conn.
Lerrick, Robert Benson, Yorktown Heights, N. Y.
Leyrer, Ralph Henry, Hamilton, Ohio
Lockhart, David A., Concord, N. C.
Lomant, Harry V., Mt. Vernon, N. Y.
Lyman, Margaret S., Iowa City.
Mitus, Anna (nee Turyczyn), Boston.
Nitowsky, Harold M., Baltimore.
Palfrey, William Gorgas, Baton Rouge, La.
Pascoe, M. Gilbert, Brantford, Ontario, Canada.
Pastel, Robert A., Syracuse, N. Y.
Raimo, Anthony S., Ridgewood, N. J.

Ready, John C., Cambridge, Mass.
Recinos, Adrian, Jr., Falls Church, Va.
Retsina-Babakitis, Mary, Chicago.
Ross, Lawrence Sidney, Huntington Station, N. Y.
Rothschild, Herbert B., New Orleans, La.
Rutledge, Lewis James, New Orleans, La.
Sceery, Robert T., Cohasset, Mass.
Sears, Edward Manning, Boston.
Smith, Fredrika P., Northampton, Mass.
Stamler, Arthur Alan, New Orleans, La.
Stone, Shirley M., New York.
Streamer, Charles Walker, Denver.
Tayloe, David T., Washington, N. C.
Vigliani, Mario, Geneva, N. Y.
Ward, George W., Philadelphia.
Watson, Robert Weir, Jr., Visalia, Calif.
Weatherly, Carl H., Boston.
Weed, Allen Stanley, Langley AFB, Va.
Whaley, Helen S., Brookline, Mass.
Wood, David W., Media, Pa.
Zang, Louis Culberson, Jr., Odessa, Texas.
Ziegler, Joseph, Burlington, N. J.
Zuckerman, Joseph I., New Orleans, La.

Specialists Certified by The American Board of Pediatrics, Inc.—The following physicians were certified by The American Board of Pediatrics, Inc., in Cincinnati on June 8, 9, and 10, 1956.

Angle, Carol Remmer, Omaha.
Baernstein, Harry Daniel, Jr., Racine, Wis.
Beverly, Bert Ira, Jr., Oak Park, Ill.
Boysen, James F., Sioux City, Iowa.
Brown, Audrey K., Detroit.
Char, Donald F. B., Philadelphia.
Chattin, William Rogers, Indianapolis.
Cimino, Louis E., Amarillo, Texas.
Cole, Wilfred Q., Jr., Jackson, Miss.
Cooper, Robert F., Columbus, Ohio.
Coverdale, Keith M., Bowling Green, Ky.
Cunnick, Dorothy Gildea, Davenport, Iowa.
DeFede, Anton John, Bakersfield, Calif.
Dickinson, David G., Ann Arbor, Mich.
Dine, Mark S., Cincinnati.
Dunn, Paul Joseph, Oak Park, Ill.
Friedman, Violet, Brooklyn.
Garber, Max J., Farmington, Mich.
Goldman, Albert, Chicago.
Gustafson, John E., West Des Moines, Iowa.
Harrison, Gunyon Mitchell, Houston, Texas.
Hartmann, Alexis F., Jr., St. Louis.
Heiligman, Emmett M., Alhambra, Calif.
Herring George W., Hattiesburg, Miss.
Heyman, Louis F., Detroit.
Hosier, Don M., Columbus, Ohio.
Howard, Robert Corlett, Denver.
Jackson, Donald W., Covina, Calif.
James, John Alexander, Dallas.
Jones, Forest Davenport, Atlanta.
Jones, John Theodore, Painesville, Ohio.
Joos, Thad H., Grosse Pointe Woods, Mich.
Kenna, Marita Dolores, Pittsburgh.
Konieczny, Leo Stanley, Ironton, Ohio.
Kopp, William Joseph, Huntington, W. Va.
Kruze, Vilis, Westchester, Ill.
Lavrich, Kathryn D., Cincinnati.
Lehmann, Walter Xavier, Wilton, Conn.
Lewis, Ray B., Columbia, Mo.
McGavran, Elizabeth Jean, Whitesburg, Ky.
MacDonald, Jack D., Portsmouth, Ohio.
Matthews, Edward C., Cincinnati.
Meyer, Maryethel, Lakewood, Colo.
Niehaus, Robert W., Cincinnati.
Nitzke, Everett A., Des Moines.
Ohren, Irving R., Milwaukee.
Oliver, Thomas K., Jr., Columbus, Ohio.
Patterson, John Robert, Kansas City, Mo.
Patton, Charles H., Jr., Brunswick, Maine.
Peterson, Shirley J., Barrington, Ill.
Phillips, Dalton T., White Plains, N. Y.
Ramsey, Bartlett W., Topeka, Kans.
Rivkin, Joseph, Mt. Clemens, Mich.
Scheip, James R., Columbus, Ohio.
Scheuber, Charles, San Antonio, Texas.
Schroeder, Robert W., Fond du Lac, Wis.
Scurletis, Theodore D., Pittsburgh.
Seabright, Howard Lee, San Diego.
Sharpsteen, J. Randolph, Jr., Man, W. Va.
Singer, Melville I., Garden Grove, Calif.
Smith, Fred Newman, Rockford, Ill.
Spencer, Hugh Burns, Tulsa.
Sternberg, Louis B., Louisville, Ky.
Stoeckle, Harry G. E., Jr., Galveston, Texas.
Suttie, Grant, DeKalb, Ill.
Sweeney, Edgar Chew, Charlotte, N. C.
Talner, Norman S., Ann Arbor, Mich.
Taylor, Paul M., Jr., Pittsburgh.
Thomas, Samuel M., Columbus, Ohio.
Thomasson, David D., Springfield, Mo.
Thompson, Arthur Lee, Detroit.
Tillou, Mary Jean, East Aurora, N. Y.
Vitanza, Constance, Binghamton, N. Y.
White, James S., Jr., Chapel Hill, N. C.
Wirtshafter, Robert J., Chicago.
Womack, Noel Catching, Jr., Jackson, Miss.
Wright, Harold L., Jr., Ann Arbor, Mich.
Zahller, F. Marshall, Omaha.
Zike, Kenneth Z., Los Angeles.

Special certificate, noncitizens:

Del Carmen, Filemon Tantoco, Manila, Philippines.
Hosseini, Mohammad Bagher, North Arlington, N. J.
James, Leonard S., Manhattan, N. Y.
Say, Mehmet Burhanettin, Nashville.
Tovar, Guillermo, Caracas, Venezuela.